MODELING THE OXIDATIVE STABILITY OF

EDIBLE FATS AND OILS IN BULK

by

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(Under the Direction of Ronald Bruce Pegg)

ABSTRACT

Lipid autoxidation is one of the central economic concerns to the edible oil and food & beverage industry. The chemistry of this degradation reaction has been understood for many decades, but successful means to predict or optimize oxidative stability have proved elusive. It is speculated here that an ongoing hindrance to these efforts has been the lack of consistency of assay selection, study design, and numerical interpretation technique among scientists attempting to quantify oxidative stability within fats and oils. This study monitored 50 samples of current commercial-use edible fats and oils for the accumulation of lipid autoxidation products according to four distinct assays throughout two months of accelerated storage. This oxidation data was examined for the derivation of a single novel comprehensive quantification of a sample's exhibition of oxidative stability. This comprehensive term was then used as the basis for the development of predictive models of oxidative stability according to numerous composition factors. Sample unsaturation (as concentrations of monounsaturated fatty acids, diunsaturated fatty acids, and triunsaturated fatty acids) demonstrated a strong correlation (R^2 = 91.5%) with the stability term, and indicated the combined presence of multiple double bonds on individual fatty acids to be associated with impaired oxidative stability. A systematic sequential approach

to model-building was then employed to negotiate the challenges of inherent redundancies within the composition variables of edible fats and oils. Independent of sample unsaturation, triacylglycerols containing one, two, and seven double bonds were positively associated with stability, and the concentrations of triacylglycerols containing three, four, five, and six double bonds were negatively associated with stability. *trans*-Fatty acids, sample purity, and α-tocotrienol were also associated with improved stability. Unsaturated fatty acids greater than 18 carbons in length and γ-tocotrienol were both associated with impaired stability. Final models including considerations of these factors were highly predictive of oxidative stability (R^2_{adj} = 97.1% for oil blends and R^2_{adj} = 96.2% for pure samples).

INDEX WORDS: lipid oxidation, edible fat, edible oil, autoxidation, mathematical modeling, unsaturated fatty acids, triacylglycerols, antioxidants, prooxidants, vitamins

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DEDICATION

This is dedicated to Taylor and Wendy, who were as sweet and patient and supportive throughout all of this as any two little kids could ever possibly be.

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CHAPTER 1

INTRODUCTION

To date, lipid oxidation remains one of the central economic concerns to both the edible oil and food & beverage industry. The oxidative deterioration of lipids in food products can affect food safety, nutrition, texture, color, as well as result in the release of aromatic volatile compounds responsible for undesirable or "rancid" flavors associated with poor food quality (Gray, 1978; Ajuyah et al., 1993; Morales et al., 1997). Lipid oxidation is in many cases the limiting factor in the shelf life of food products, and thus, is one of the key elements that require consideration in a product's design, formulation, processing, packaging, and storage (Chaiyasit et al., 2007).

A fairly sophisticated understanding of the mechanisms of lipid oxidation had been achieved by the 1940s. However, despite this fact, there seems to exist a large number of confounding variables that can affect lipid oxidation in bulk lipids, and perhaps even more so in food systems. Thus, the means by which to accurately predict lipid oxidation have proved elusive, as too have consistent methods of quality control (Lea and Hawke, 1951; Min and Boff, 2002). Many contributing factors to lipid oxidation have been cited including: fatty acid composition (particularly the degree of unsaturation), storage temperature, processing methods, and the concentrations of oxygen, free fatty acids, pro-oxidants, and antioxidants. The interaction of these factors (as well as matrix effects) has not been well understood on a quantitative level (Choe and Min, 2006; Chaiyasit et al., 2007). Even when focusing only upon a single variable of lipid oxidation within a single lipid source, studies have often failed to find

consistent relationships. For example, higher antioxidant concentrations in bulk oils do not necessarily directly correlate with higher lipid stability (Satue et al., 1995; Baldioli et al., 1996). Further, in the case of emulsions, studies on lipid oxidation need to take into account the heterogeneous physical properties inherent to the lipids studied and the role of these micro-environments upon their oxidative stability (Ghosh and Tiwary, 2001).

It is important to note that the term 'oxidative stability' is a fairly nonspecific term that refers to the exhibited resistance of a lipid or lipid-containing food product to undergo oxidation (Guillén and Cabo, 2002; Velasco and Dobarganes, 2002). This level of resistance may be determined according to a multitude of methodologies and statistical interpretations. For example, it may be presented as the time period preceding particular levels of accumulation of known oxidation products (*e.g.* hydroperoxides) in challenged edible oils, the quantified magnitude of said oxidative end-products, or the time period preceding the rancidification of the oil as determined by a sensory panel, to name a few.

Today there are still only a limited number of approaches used to retard lipid oxidation, and even these have been of diminished commercial viability in the marketplace due to shifting consumer demands and market trends (Joppen, 2006; Chaiyasit et al., 2007). Among the most historically common and effective methods of control has been the preferential use of lipids with higher proportions of saturated fatty acids in food products - as these molecules are not susceptible to lipid oxidation reactions. Commercial hydrogenation procedures, which yield more highly saturated oils from unsaturated ones, has also been used as a means to improve oxidative stability. Such practices are now of less practical use due to their being in opposition with current nutritional recommendations, which call for the consumption of higher proportions of unsaturated fatty acids and reduced consumption of saturated or *trans*-fatty acids (Radke,

2008; Abe et al., 2009; Benjamin, 2011). Similarly, the use of effective synthetic antioxidants (*i.e.* BHA, BHT, TBHQ, etc.) has fallen into disfavor in recent years due to consumer concerns of possible health risks of these compounds and because their use prevents the product from achieving the increasingly important "clean label" (Joppen, 2006; Brewer, 2011). These trends have increased the necessity to attain a more sophisticated understanding of what factors most affect the oxidative stability of lipids and what can be done to achieve optimized formulations.

Currently, the scientific literature depicts only infrequent attempts to develop mathematical relationships capable of predicting oxidative stability in bulk fats and oils. The study described in this report monitored the rate and extent of lipid oxidation within 50 edible oils and fats according to four distinct methods of assessment. These methods evaluated the accumulation of both primary and secondary products of lipid oxidation, providing a telling and thorough picture of the stability of the lipids assayed.

The obtained oxidative stability data is first used to develop a meaningful quantitative formula by which to less ambiguously define "oxidative stability" (chapter 3). This numerical term of oxidative stability is then compared mathematically to concentrations of unsaturated fatty acids (chapter 4), and then to more detailed data regarding the chemical composition of the oils. Many of these factors are highly redundant, which presents a challenge regarding the meaningful interpretation of their possible unique effects. The strategy for overcoming this challenge is described in chapter 5, in which the justification for the ordering of considered factors is also discussed. Chapters 6 and 7 then proceed with the strategy outlined in chapter 5 and show the systematic improvement of the model according to the sequential consideration of the following factors: (1) triacylglycerol double bond distribution, (2) fatty acid data, (3) sample purity, and (4) endogenous vitamin concentration.

The objective of this study is to more meaningfully define oxidative stability numerically, to provide insight into the contributing factors influencing oxidation rates of oils and fats, and to produce models of practical use for ingredient selection as well as in the possible optimization of edible oil and fats.

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CHAPTER 2

LITERATURE REVIEW

Lipid Oxidation: Principles, Mechanisms, and Products

The term lipid oxidation is a general classification for chemical interactions occurring between reactive oxygen species and unsaturated fatty acyl groups, and can include a large variety of specific chemical reactions and products (Frankel, 2005). The process of lipid oxidation in refined edible fats and oils is generally viewed as consisting of the two subcategories of autoxidation and photooxidation (Choe and Min, 2006). The distinction between autoxidation and photooxidation lies in the dissimilarity of the environmental variables required for their occurrence, as well as the different possible electron orbital states of molecular oxygen that are present in the two different reaction mechanisms (Min and Boff, 2002). These different orbital states of molecular oxygen are defined as triplet oxygen ($^3O_2$) and singlet oxygen ($^1O_2$), respectively.

In edible oil production, the occurrence of photooxidation is more easily controlled than autoxidation, and therefore is of significantly less concern to the food industry (Choe and Min, 2006). Nevertheless, a comprehensive understanding of lipid oxidation in bulk lipids requires the consideration of photooxidation in addition to the broader subcategory of autoxidation. Therefore, the different orbital states of molecular oxygen and photooxidation are briefly discussed below before expanding on autoxidation theory.

Triplet and Singlet Oxygen

Molecular oxygen (consisting of two covalently bound oxygen atoms) in its most stable form occurs in a 'triplet' state, for which the molecular orbital configuration is shown in **Figure 2.1**. This triplet state is defined by the occurrence of the two unpaired electrons occupying the π^* molecular orbitals. This configuration is distinct from that of most stable diatomic molecules in that it exists in a diradical state; a fact which provides some explanation of molecular oxygen's unique degree of reactivity. This diradical state would generally be considered energetically unfavorable, but in the case of molecular oxygen, this configuration is the most stable one (Hongo et al., 2004; Van, 2007).

Molecular oxygen occurring in a 'singlet' state is shown in **Figure 2.2**. Here the electrons within the π^* molecular orbitals are paired so as to preclude the presence of free radicals, but their spin configuration violates Hund's rule. Hund's rule states that free single electrons must populate electron orbitals of equal energy before they are paired with electrons of opposite spins. As a result of this singlet state of molecular oxygen, there exists an inherent electronic repulsion in its π^* molecular orbital; increasing the species' reactivity (Darvesh and Boyd, 1989). This singlet state is energetically highly unfavorable (representing an energy state a minimum of 22.4 kcal above the triplet ground state), very electrophilic, and readily reactive with other singlet state molecules. This is especially the case concerning the reaction of singlet oxygen with singlet state unsaturated fatty acids (Min and Boff, 2002; Schweitzer and Schmidt, 2003).

The formation of singlet oxygen from triplet oxygen can occur due to chemical processes, enzymatic actions, gaseous discharges, and the decomposition of hydroperoxides (Khan and Martell, 1967; Rosenthal, 1985). In food systems, the majority of singlet oxygen is formed as a result of the interaction of light, photosensitizers, and triplet oxygen (Clements et al., 1973).

Pigments such as chlorophyll, hematoporphyrins, and riboflavin are commonly found in food and can serve as efficient photosensitizers (Bradley and Min, 1992). These compounds contain conjugated double bond moieties, which absorb visible light energy and this energy input in-turn pushes an electron within the compound to a higher energy state (*i.e.*, "excited singlet state") (Min and Boff, 2002). Proceeding *via* an intersystem crossing mechanism, the excited singlet sensitizer may then be converted to an excited triplet sensitizer (Wilkinson et al., 1993). This excited triplet sensitizer then degrades to the singlet ground state by a release of energy; the result of which may convert triplet oxygen to singlet oxygen (Van, 2007).

Photooxidation

Photooxidation (also referred to as "photosensitized oxidation") specifically describes oxidation of unsaturated lipids in the presence of the highly reactive singlet oxygen species (Carlsson et al., 1976). A depiction of the reaction between singlet oxygen and linoleic acid is shown in **Figure 2.3**. Due to spin conservation, singlet state unsaturated lipids are not favorably reactive with the ground-state triplet oxygen. However, the reaction between an unsaturated fatty acid and singlet oxygen requires no input of energy, and can occur at a very rapid rate (on the order of $10^4 – 10^5$ $M^{-1}s^{-1}$) (Doleiden et al., 1974).

Mechanistically, the singlet oxygen molecule simultaneously reacts with the α-olefinic carbon and abstracts the γ-allylic hydrogen, creating a concerted six-membered ring, which can result in the formation of hydroperoxides (Korycka-Dahl and Richardson, 1978). As shown in **Figure 2.3;** the hydroperoxide formed from photooxidation of linoleic acid can either be conjugated or unconjugated, providing an important distinction from the hydroperoxides produced by autoxidation (*i.e.* which are exclusively conjugated; Thomas and Pryor, 1980).

Also note that in the case of this example, this reaction can occur on the double bond at the 12 position as well as the 9 position as shown (Frankel, 1985). Once formed, hydroperoxides can undergo a process of decomposition steps resulting in volatile compounds detrimental to product quality (Popov and Yanishlieva, 1967; Evans et al., 1969). These decomposition steps are a common trait of both mechanisms of lipid oxidation, and will be addressed in more detail in our discussion of autoxidation.

As aforementioned, the reaction of unsaturated fatty acids with singlet oxygen is autocatalytic and can proceed at a rapid rate. **Figure 2.4** depicts a comparison of the relative reaction rates of unsaturated fatty acids with both triplet and singlet oxygen. As shown in **Figure 2.4;** reaction rates with singlet oxygen are often faster (Gunstone, 1994; Min and Boff, 2002). This fact; however, is counterbalanced in food industry by the nature of edible oil production. Compounds capable of acting as photosensitizers are effectively removed during the oil refining process. Further, the exposure of sensitive oils to ultraviolet/visible electromagnetic radiation can be retarded by proper packaging materials and storage. This results in a minimization of the likelihood for photosensitized oxidation being a contributing factor in the oxidative degradation of commercially refined edible fats and oils (Jung et al., 1989; El-Shattory et al., 2005).

Autoxidation

Autoxidation does not depend on the formation of singlet oxygen, and instead begins with the conversion of a fatty acid or acylglycerol into a radical state *via* the removal of a hydrogen atom. This initial transition of fatty acid into alky radical is known as “initiation”, and represents

the first step in the sequence of reactions of autoxidation. This sequence is generally characterized as follows (adapted from Kanner et al., 1987; Nawar, 1998):

Initiation	The formation of free radical species.
Propagation	The chain reactions involving the free radical species.
Termination	The formation of non-radical products.

The chemical reactions of these stages can then be summarized as follows (R represents a lipid alkyl chain; adapted from Choe and Min, 2006):

Initiation	RH	→	R· + H·	(1)
Propagation	R· + $^3O_2$	→	ROO·	(2)
	ROO· + R'H	→	ROOH + R'·	(3)
	RO· + R'OOH	→	ROO· + ROH	(4)
	RO· + R'H	→	ROH + R'·	(5)
Termination	ROO· + R'·	→	ROOR'	(6)
	R· + R'·	→	RR'	(7)
	RO· + R'O·	→	ROOR'	(8)
	RO· + R·	→	ROR'	(9)

This initial conversion of singlet state lipid RH into the free radical R· in reaction (1) is fundamental to the occurrence of autoxidation, and its mechanism is still not fully understood (Min and Boff, 2002). This reaction can theoretically occur in an autocatalytic manner, but much work has been done to determine factors capable of either triggering or promoting the degradation reaction (Kubow, 1992; Frankel, 2005). Throughout a great number of studies, initiation has been associated with the action of heat, light, certain enzymatic reactions, acidity, ozone, nitrogen dioxide, pressure, and others (Khan, 1955; Penning et al., 1996; Morita and Tokita, 2006; Musialik et al., 2008; Pryor et al., 1980; Neuenschwander and Hermans, 2010). The presence of metal catalysts have received much attention as a possible enacting agent of

initiation of autoxidation, which may occur *via* the mechanism RH + M^{3+} → R· + H^{+} + M^{2+} (Min and Boff, 2002).

There has also been a longstanding attribution of the initiation reaction (1) to the presence of hydroperoxides, which are therefore at once both a primary product of oxidation as well as a compound often considered centrally responsible for radical generation (Morita and Tokita, 2006). The role of hydroperoxides as positively affecting initiation rates has been supported by studies, and it provides a possible explanation for the exponential increases of initiation rate that is often observed over time (Chaiyasit et al., 2007; Kim et al., 2007). There does exist, however, a minority opinion which challenges the belief that hydroperoxides are centrally responsible for autocatalytic radical generation (Morita and Fujimaki, 1973; Morita et al., 1976). Morita and Tokita (2006) recently investigated the question experimentally and concluded that main-product hydroperoxide has little autocatalytic radical-generating activity at room or body temperature.

As a result of the initiation reaction (1), alkyl radicals are formed on the unsaturated lipid molecule. The free radical electron present on the alkyl group then delocalizes over the double bond system and results in a double bond shift. In the case of polyunsaturated fatty acids, this shift causes the formation of conjugated double bonds (meaning the double bonds are not interrupted by a methylene carbon). An example of this process occurring on linoleic acid is depicted in **Figure 2.5**; note the different end locations of the radical electron that can result from the described delocalization. The relative occurrences of radical electron location resulting from the autoxidation of oleic acid, linoleic acid, and linolenic acid are shown in **Table 2.1**.

Following free radical mediated initiation (Eq. 1) of autoxidation, the oxidation pathways and mechanisms of subsequent reactions are fairly well characterized in the literature. A radical propagation reaction (2) follows in which triplet oxygen reacts with the alkyl group generated in

intiation to form a highly reactive peroxy radical (ROO·). This peroxy radical then in turn abstracts hydrogen from another unsaturated fatty acid and produces a hydroperoxide and a new alkyl radical as shown in reaction (3) (Chaiyasit et al., 2007). The formation of a hydroperoxide from one of the possible alkyl radicals which can result from linoleic acid is shown in **Figure 2.6**. These hydroperoxides are classified as "initial" or "primary" oxidation products and often serve as a crucial indicator of the current oxidative state of oils and fats. The accumulation of hydroperoxides over time can also be monitored in order to determine the oxidative stability of lipids. It is important to note that hydroperoxides are not sensorially active. It is only once they undergo decomposition into smaller aromatic compounds and associated fragments that they are perceived and thus can compromise product quality.

The most likely decomposition pathway of hydroperoxides is that of homolytic cleavage between the two bound oxygen atoms (ROOH → RO· + ·OH), resulting in an alkoxyl radical and a hydroxyl radical (Min and Boff, 2002). This alkoxyl radical is then cleaved *via* β-scission of a carbon-carbon bond that can occur on either side of the bound oxygen. The potential products of these reactions (known as "secondary" products) are numerous, and their relative formations are dependent upon the initial reactants, additional reacting compounds, and the specific atomic locations of the steps of decomposition and cleavage (Min and Bradley, 1992; Chaiyasit et al., 2007). **Figure 2.7** illustrates the decomposition of a hydroperoxide molecule. **Table 2.2** lists the many potential decomposition products resulting from the β-scission reaction outlined in **Figure 2.7**.

Effect of Autoxidation on Edible Oil Sensory Quality

As stated above, there are many possible outcomes of the autoxidation of mono- and polyunsaturated fatty acids. Due to the series of decomposition reactions occurring in lipid oxidation, many of the resulting compounds will be short-chain fatty acid derivatives; a large proportion of which will be volatile and capable of contributing off-flavors to oils and fats (Min and Boff, 2002). Further, the accumulation of these volatile aromatic compounds often serves as a limiting factor in the shelf-life of lipids (Choe and Min, 2006).

In assessing the potential impact of lipid oxidation products upon sensory quality, it is often useful to note both the perceived flavor associated with the end products as well as their sensory threshold values. It is important to recognize that the most abundant oxidation products of a given lipid will not necessarily be that which are of the greatest concern in regards to lipid quality. There can exist great differences in the partitioning of different volatile lipid decomposition products into the headspace of a food product, as well as differences in the relative tendency of those compounds to be perceived by the consumer. Further, different compounds can impart different off-odors and flavors that can affect the lipid's perceived quality. Sensory threshold values for many common end-products of lipid oxidation are summarized in **Table 2.3** (Frankel, 1985). Some common sensory descriptors attributed to oxidized lipids, and their characteristic volatiles, are shown in **Table 2.4** (Shahidi and Zhong, 2005). Due to their frequently high concentrations and their relatively low threshold values, aliphatic carbonyl compounds are generally considered to have the greatest influence on oxidized oil flavor (Choe and Min, 2006).

Note that the values both for flavor notes and for sensory thresholds have traditionally been determined *via* the assessment of oxidative products in isolated solutions (*e.g.* in pure water or

oil). In the case of real food systems, the sensory effects of these compounds have been shown to vary widely due to their interaction with one another as well as with other food components (*i.e.* as in "masking" or working synergistically; Venkateshwarlu et al., 2004). As such, the actual effects of lipid oxidation on sensory perception are difficult to accurately ascertain strictly *via* chemical analysis, and thus, tandem sensory analyses are often necessary.

Factors Affecting Autoxidation Rates

Many factors affect the rates of the autoxidation in bulk refined lipids. These factors can be grouped into two distinct categories: those which are intrinsic to the lipids (*e.g.* fatty acid composition), and extrinsic factors (*e.g.* storage conditions). Intrinsic factors affecting lipid stability that are most often cited in the literature include the fatty acid composition of the oil as well as the internal concentrations of free fatty acids, mono- and diacylglycerols, phospholipids, pro-oxidants (heavy metals), and antioxidants (Choe and Min, 2006). Commonly cited extrinsic factors include processing parameters (*e.g.* chemical vs. physical refining and thermally induced process contaminants), external oxygen availability (typically a factor in processing and packaging), added antioxidant stabilizers, and storage conditions (Choe and Min, 2006; Chaiyasit et al., 2007). In many cases these cited factors can overlap with one another based on their capabilities to elicit/inhibit lipid oxidation and interactions thereof. This fact makes it difficult to distinguish or quantify the effect each individual factor may have upon lipid oxidation rates; and thus, a more all-inclusive approach is necessary.

A key example of the pitfalls encountered in monitoring the oxidative stability of oils based on their composition has to do with variations in the practice of edible oil refining. In fact, it has been proven that two different refined sesame oils, ostensibly of the same crude oil

composition, can exhibit dissimilar oxidative stability (Abou-Gharbia et al., 2000). Further, processing can elicit a variable effect upon the retained inherent antioxidant contents of edible oils (Sherwin, 1978). For example, it has been shown that soybean oil has a higher oxidative stability in its crude form than after being refined; speculated to be due to the loss of antioxidants in the refining process (Jung, 1992).

Intrinsic Factors

Fatty Acid Composition

The level and rate of oxidation experienced by a challenged lipid sample is generally in positive correlation with a greater degree of fatty acid unsaturation. This seems logical given that oxidative reactions initiate upon carbon-carbon double bonds (Parker, 2003). In most cases, higher proportions of unsaturates inherent to lipids will lead both to more rapid autoxidation as well as to a greater accumulation of lipid oxidation products (Martin-Polvillo et al., 2004). Min and Bradley (1992) compared the relative autoxidation rates of oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids and determined the relative rates to be 1:40 to 50:100. This study was based on monitoring the oxygen uptake of the lipids during experimentation. In 1973; it was established that the hydrogenation of the mono- and poly-unsaturates within soybean oil to more saturated fats resulted an improved oxidative stability of the oil (Evans et al., 1973). Similarly, in, 2002 it was determined that the minimization of double bonds within an oil through the use of gene silencing upon the parent plant also proved effective in improving the oil's oxidative stability (Liu et al., 2002).

Given this observed correlation, the simple measure of Iodine Value (a measure of the quantity of double bonds present) of lipids can often serve as a fair, yet still not entirely

consistent, indicator of the lipids oxidative stability. In, 2002; Tan et al. analyzed 12 oil samples for a variety of traits including Iodine Value (IV) and Oxidative Stability Index (OSI, a measure of an oil's resistance to oxidation; a higher value denotes a higher stability). The results of this study are plotted in **Figure 2.8** and **Figure 2.9**. **Figure 2.8** shows the OSI data plotted as a function of the IV data including all the oil samples tested. Of course, one would expect an inverse association between OSI values and IV; however, this wasn't the case. Therefore, the authors removed the oils that were statistical outliers (*i.e.* likely due to refining or the addition of antioxidant stabilizers, etc.), and the resulting graph is depicted in **Figure 2.9**. When viewed together, **Figure 2.8** and **Figure 2.9** exemplify the point that the higher the level of fatty acid unsaturation in bulk oils (increasing IV), the lower the inherent oxidative stability (decreasing OSI). Further, without consideration of other variables such as processing and the inclusion of antioxidant additives, there is clearly too much inconsistency for this relationship to be considered accurately predictive.

Free Fatty Acids, Mono- and Diacylglycerols, and Phospholipids

The vast majority of fatty acids in refined edible oils are ester-bound in the form of triacylglycerols. This is already inherently the case for most crude oils; and is accentuated by the refining process. Mono- and diacylglycerols and phospholipids are largely removed from lipids during the degumming step of most edible oil refining given their moderately polar character. Further, free fatty acids are stripped off in commercial deodorization towers and serve as a quality assurance indicator of the refining process (Dijkstra and Segers, 2007). Despite these considerations, the minor presence of free fatty acids, mono- and diacylglycerols, and phospholipids have all been shown to exhibit effect upon the rate of autoxidation (Mistry and

Min, 1987; Kim and Choe, 2005; Zhang et al., 2007). Thus, it is important that levels of such components are minimized in bulk refined oils and fats.

The presence of free fatty acids (FFA) in edible oils and fats has been shown repeatedly to have a positive correlation with their rate of autoxidation (Kinsella et al., 1978; Handel and Guerrieri, 1990; Frega et al., 1999). Paradiso et al. (2010) recently published a study on the effects of increased levels of FFA in olive oil and the resultant effects upon oxidative stability of the oil. Therein, a slightly positive correlation was observed at levels of FFA between 0-1%, above which yielded a dramatic decrease in oxidative stability.

An explanation of the effect of FFA on a lipid's oxidative stability lies in their capability of acting as pro-oxidants in an oil system; both by engaging directly in chemical pro-oxidative reactions as well as by altering the physical properties of the system (Miyashita and Takagi, 1986; Paradiso et al., 2010). The above mentioned direct action of FFA is attributed to their carboxyl group, which has been demonstrated to have a catalytic effect on the decomposition of hydroperoxides. That is, FFA may reduce the stability of the primary products of lipid oxidation, thus hastening the development of secondary oxidation products and the potential initiation of further free-radical mediated oxidation reactions. The effect of FFA on the physical properties of lipids is due to their amphipathic nature (possessing both hydrophobic and hydrophilic regions), which favors their conglomeration both upon the surface of the oil as well as upon the surface of reverse micelles formed interior to the oil (Min and Mistry, 1987; Waraho et al., 2009). This convergence of FFA upon the oil surface reduces surface tension and is believed to increase the rate of diffusion of oxygen into the oil and thereby accelerate autoxidation (Choe and Min, 2006). Further, it is speculated that the accumulation of FFA at the surface of reverse micelles serves to attract cationic transition metals, which are therefore in a location more

conducive to their interaction with lipids for the promotion of oxidation as a whole (Waraho et al., 2009).

In addition to FFA, studies have also shown that the increased presence of mono- and diacylglycerols in oils and fats can correlate with decreased oxidative stability (Mistry and Min, 1988). Mono- and diacylglycerols possess hydrophilic hydroxyl groups on their glycerol backbone in addition to hydrophobic hydrocarbon chains; and are therefore amphipathic (Armand et al., 1996). Their inclusion in edible oils can influence the oil's surface tension and the properties of formed reverse micelles, operating in a manner very similar to that described above for FFA (Mistry and Min, 1988; Choe and Min, 2006). It is important to note that mono- and diacylglycerols are effectively limited by the refining process in most instances, resulting in an increased stability of oils and fats post refining. Palm oil is a special case; however, in which diacylglycerols can be quite high (4-12%, with a mean of ~6.5%) as a byproduct of triacylglycerol synthesis in the plant (Siew, 1995; Siew and Ng, 1999; Long et al., 2005).

Phospholipids are present in crude oils, but are almost entirely removed during the refining process (one study determined the reduction to be approximately 99.9%; Yoon and Min, 1987). Those which remain in edible oils have been shown to potentially serve as both pro-oxidants and antioxidants. This behavior is believed to depend upon the type and concentration of phospholipids as well as upon the concentration of pro-oxidants in the oil. The pro-oxidant effect of the inclusion of phospholipids in edible oils is believed to be due to the same phenomenon observed in the case of FFA, mono- and diacylglycerols; their amphipathic nature (Rosenberg and Ron, 1999). An antioxidant effect; however, has been observed when phospholipids are in the presence of Fe^{2+}. A hypothesis exists that phospholipids can exhibit antioxidative action by sequestering metals at the micellular interface in bulk oils, thereby

inhibiting the metal's ability to act as a pro-oxidant (Yoon and Min, 1987; King et al., 1992). This antioxidant activity has been observed to achieve maximum effect at phospholipid concentrations of between 3 and 60 ppm; concentrations well beyond the magnitude expected to remain in oils and fats post refining.

Pro-oxidants

Pro-oxidant is a broad term that can describe any compound which initiates, facilitates, or accelerates lipid oxidation; the actions of which may occur by multiple different mechanisms. Pro-oxidants can increase the rate of oxidation either through direct interaction with fatty acids, by contributing to the formation of free radicals, or by altering the physical properties of the lipid matrix (as discussed above in the case of FFA). In many cases, compounds which can serve as pro-oxidants in one environment may also operate as antioxidants in another environment (or vice-versa), or simply elicit no effect at all (Aruoma et al., 1996; Decker, 1997). This section will address some of the key compounds that can elicit pro-oxidative effects in edible oils.

The presence of heavy metals in edible oil can increase the rate of oxidation by a variety of means. Metals have been shown to significantly decrease the activation energy required in the initiation stages of autoxidation (Eq. 1 above), and also to produce lipid alkyl radicals through a direct reaction with fatty acids (Jadhav et al., 1995). Each of these effects leads to the proliferation of primary oxidative products (*i.e.* hydroperoxides), but metals are known to also promote the decomposition of hydroperoxides as well, operating by the following proposed mechanism (10) (adapted from Benjelloun et al., 1991).

Decomposition $ROOH + Fe^{2+} \rightarrow Fe^{3+} + OH^- + RO\cdot$ (10)

Therefore, the presence of metals can increase the rate of initiation of free-radical mediated autoxidation, and may also directly increase the rate at which secondary oxidation products are formed throughout the decomposition of hydroperoxides. Copper (especially cuprous Cu+; Lauritzsen et al., 1999) has been found to accelerate the rate of oxidation by the greatest magnitude, followed by ferrous iron (Fe^{2+}), and then ferric iron (Fe^{3+}; Mei et al., 1998).

Metals have also been shown to indirectly increase an oil's susceptibility to autoxidation by interacting with antioxidant compounds present in the oil and negating their ability to slow or hinder oxidation in a system. For instance, Keceli and Gordon (2002) demonstrate that ferric iron can decompose phenolic compounds (a prominent family of antioxidants) and therefore can decrease the oxidative stability of edible oils. It is important to note that the presence of metals in edible oils is typically reduced a great deal by the refining process, but may still remain in refined edible oils in quantities sufficient to affect autoxidation (Sleeter, 1981). Heavy metal content can be of particular concern in unrefined edible oils, such as extra virgin olive oil (Choe and Min, 2006); however, these oils in many cases also contain a higher amount of inherent antioxidants.

Antioxidants

The term antioxidant describes compounds which inhibit, prevent, or slow lipid oxidation (Sies, 1997). Like the term pro-oxidant, the term antioxidant can describe many distinctly different compounds, reactions, and modes of function. A wide range of antioxidants have been determined to be naturally present in edible oils. Natural or synthetic antioxidants can also be added to oils post refining, but those of natural origin are generally perceived much more favorably by the modern consumer (Chaiyasit et al., 2007). Synthetic antioxidant stabilizers will

be discussed in more detail below. The most common categories of natural antioxidants in foods include phenolic compounds (including the ubiquitous tocopherols), ascorbic acid, carotenoids, and sterols (Choe and Min, 2009). Examples of the chemical structures of natural antioxidants appear in **Figure 2.10**.

Antioxidants can be classified either as "primary antioxidants" (those which actively inhibit oxidation reactions) or "secondary antioxidants" (those which inhibit oxidation indirectly, by mechanisms such as oxygen-scavenging, binding pro-oxidants, etc.) (Reische et al., 2002). Primary antioxidants operate by donating a hydrogen atom to free radicals and becoming a more "stable" radical form themselves. **Figure 2.11** shows such a phenomenon exhibited by phenolic antioxidants; termed conjugative resonance stabilization. A primary antioxidant's efficacy is dependent upon it having a lower reduction potential than the free radicals present in the system as well as its ability to yield low energy radicals that will not react rapidly with unsaturated fatty acids (Chaiyasit et al., 2007). The most efficient primary antioxidants are those capable of resonance delocalization of radicals formed post reaction with free radicals (Shahidi et al., 1992), similar to the mechanism shown in **Figure 2.11**. Phospholipids, on the other hand, are an example of a secondary antioxidant. As discussed above, phospholipids can slow the oxidation of lipids by interfering with the ability of metals to act as pro-oxidants in certain systems.

A phenomenon has been demonstrated in recent years in which more polar antioxidants have higher efficacy in less polar environs and vice-versa (Shahidi, 2011). This phenomenon has been appropriately dubbed the 'Polar Paradox.' In the case of bulk oils, this effect had long been speculated to be attributable to a conglomerative action occurring at the air-oil interface. This notion; however, is inconsistent with the fact that air is less polar than oil and has lost merit in recent years (Chaiyasit et al., 2008). More recent work suggests that there exists a distribution of

small reverse micelles in bulk oils (*i.e.* consisting of water in the interior) and antioxidants may exhibit their antioxidative action at the interface of these micelles (Decker et al., 2005; Chaiyasit et al., 2007). This development not only has implications for the efficacy of antioxidants (depending upon their ability to interact at this water-lipid interface), but also suggests that these reverse micelles may be of major significance to the oxidation in oils in bulk and also in emulsions. A better understanding of this water-lipid interface could be crucial to the development of better methods of quality control in the edible oil industry.

Extrinsic Factors

Oil Processing

The two main thermal processes in which oils are submitted to relatively high temperatures (T > 160 °C) are deep-frying and deodorization. Thermal treatment of lipids can trigger the formation of undesired compounds such as *trans*-fatty acids (Wolff, 1993), cyclic fatty acid esters (Destaillats and Angers, 2005), and acylglycerol polymers (Beljaars et al., 1994); the concentration of which has been shown to positively correlate with the rate of autoxidation (Yoon et al., 1988). Deep-frying can result in the formation of thermo-oxidized products and polymers through the reaction of lipids with water, oxygen, and various components of food matrices (Batista & Sanchez-Muniz, 2001). Sheehy et al. (1994) determined that such thermally-induced oxidation products can even neutralize the antioxidant effects of α-tocopherol (Sheehy et al., 1994).

Oxidation Products

Primary or secondary oxidation products (*i.e.* especially hydroperoxides and their decomposition products) have been proven to act as pro-oxidants in lipid systems. As previously discussed, this effect can potentially be attributed to the tendency of hydroperoxide decomposition products to be amphipathic, and therefore, lower the surface tension at the air-oil interface thereby increasing the diffusion rate of oxygen into the lipid system (Choe and Min, 2006). Further, this can also be the case in the more recently proposed 'Polar Paradox' phenomenon. Therein the lipid oxidation products may reduce the surface tension at the oil-water interface of the reverse micelles where antioxidants and pro-oxidants can exhibit their proposed action (Chaiyasit et al., 2007).

Added Antioxidant Stabilizers

The use of added natural (*e.g.* antioxidant: extract of rosemary E-392; containing carnosic acid or carnosol) or synthetic (*e.g.* butylated hydroxyanisole [BHA-E320], butylated hydroxytoluene [BHT-E321], propyl gallate [PG-E310], and *tert*-Butylhydroquinone [TBHQ-E319]) antioxidants has been shown to be among the most effective methods of controlling lipid oxidation in bulk. Further, antioxidants may interact with each other to produce additive, synergistic, and even antagonistic effects (Choe and Min, 2009). Antioxidants can also exhibit pro-oxidative effects in certain circumstances (most often as a result of being present in excessive concentrations), thereby making the indiscriminate mass addition of antioxidants to a system a possibly ineffective and potentially detrimental practice.

Storage Conditions

Increases in the temperature applied to oils have been shown to correlate with an increase in the rate of oxidation of those oils (Vercellotti et al. 1992). This effect is often attributed to the simple input of sufficient activation energy to support the initiation of the oxidation of lipids. Heat has also been shown to potentially degrade antioxidants as well as produce pro-oxidants in lipid systems, and thus, can further affect their oxidative stability (Sheehy et al., 1994; Shahidi and Spurvey, 1996; Lee, 2007).

Crapiste et al. (1999) monitored the oxidation rates of sunflower oil when held at 30 °C, 47 °C, and 67 °C. Therein the authors measured the accumulation of hydroperoxides *via* the peroxide value (PV) test; the results are summarized in **Figure 2.12**. PV will be discussed in more detail below under the section on the assessment of oxidative stability. As seen in **Figure 2.12,** a marked increase in the rate of formation and magnitude of accumulation of hydroperoxides was exhibited in the oils with respect to increased storage temperature. It is also important to note that the authors observed a marked increase in FFA over the course of storage in the case of the oil sunflower oil stored at 67 °C, but not for the oil at 30 °C or 47 °C. The increased prevalence of FFA in the most thermally challenged sample (67 °C) could have served as a partial contributing factor to its increased oxidation rate when compared to the other two samples (30 and 47 °C).

Lee (2007) studied the effects of temperature on the oxidation of soybean, sunflower, and olive oil. Results of this study showed that all three edible oils exhibited significant positive linear relationship between oxidation rates and storage temperature. Within the evaluated temperatures (25 °C – 80 °C), the relationship was summarized by the authors according to the following equation (11):

Oxidation Rate $\log \tau = E_a/2.303R(1/T) + \text{constant}$ (11)

(where, τ, E_a, R, and T are equivalent to the ionization potential, activation energy, universal gas constant, and absolute temperature, respectively).

Oxygen Concentration

Given that oxygen plays such a key role in the autoxidation of lipids, when oxygen is only present at low concentrations it may serve as a limiting factor in the reaction. Several studies have shown the concentration of oxygen can reach levels as high as 4-10% before oxidation rates cease to be dependent upon oxygen concentration (Kacyn, 1983; Andersson, 1999). Oxygen concentrations can depend upon the volume and surface area of the oil, the integrity of the package containing the oil, the levels of oxygen exposure during processing steps, the degree of mixing of the oil, and the oil type (Choe and Min, 2006). Due to this fact, when typical vegetable oils come out of commercial deodorization towers (*i.e.* ultra low concentrations of dissolved gasses) they are gassed with nitrogen and immediately packed (Dijkstra and Segers, 2007).

Oxidative Stability: Assessment, Interpretation, and Modeling

Oxidative stability is not an intrinsic trait of a food product, but rather, it includes the effects of all relevant factors both internal and external to that product, and can change significantly throughout a product's life-cycle (Chaiyasit et al., 2007). Therefore, any assay designed to evaluate the oxidative stability of fats and oils requires the measurement of changes in the lipids over time.

A long used and tested means by which to measure the oxidative stability of lipids in commercial situations is a storage test. Therein, the lipids are stored in a manner resembling real-world situations and the proliferation and/or decline of certain oxidation 'markers' are measured by chemical and sensory assays in real-time. Given the costs associated with the storage of large volumes of food products and the time needed to run such tests (*e.g.* if a food product is being examined for a 1-3 year shelf-life), accelerated storage tests are often employed to reduce resources input (O'Keefe and Pike, 2010). Of course, a fully comprehensive study of lipid oxidation would require the continual assessment of all known products of primary and secondary lipid oxidation over long periods of time, coupled with full descriptive sensory analysis, but this also is impractical. To this end, the study of chemical lipid oxidation needs to be re-evaluated with consideration of the multiple factors responsible for the oxidative degradation of lipids.

Accelerated Methods

Methods designed to determine oxidative stability in an accelerated timeframe may do so according to two very different approaches. One approach is that of a rapid test, which involves exposing oil and fat samples to extreme conditions for short periods of time and monitoring their degradation (*e.g.* the Rancimat Test or 'Oil Stability Index' as well as the Oxygen Bomb Method, to be discussed below). The other approach is that of an Oven Storage Test, a method which helps to mildly accelerate the oxidation of the lipid samples, while monitoring changes in their oxidative state over time.

Oil Stability Index

The Oil Stability Index (OSI) involves the use of elevated temperatures (100-120 °C) and infused air to release acidic volatiles from a lipid sample, which are then captured in deionized water and result in a measured change in conductivity (Jebe et al., 1993). Although very rapid, there has been increasing concern in recent years that the usefulness of such tests is limited by the possibility that the high temperatures employed therein may alter the decomposition of the lipid samples (O'Keefe and Pike, 2010). Specifically, the additional kinetic energy results in the formation of compounds that do not form in samples held at lower temperatures. Another concern highlighted recently by Farhoosh (2007) in the case of the OSI, is that of the high variability in OSI results due to simple differences in the operational parameters employed in the assay. In its favor, OSI results have in some cases demonstrated strong correlations with the shelf-stability of products as evaluated by sensory analysis (Coppin and Pike, 2001).

Oxygen Bomb Method

The oxygen bomb method (OBM), like the OSI, is a rapid test for the oxidative stability of lipids. Unfortunately; however, like the OSI it runs the risk that the small changes in procedure can greatly affect the results obtained (Frankel, 1993). The OBM involves the measurement of the uptake of oxygen from a lipid sample while put under high pressure. It has been suggested that this method may show a better correlation with rancidity shelf life tests than OSI, but inter-laboratory studies have demonstrated an unacceptable degree of variation in results obtained using the OBM (Kurtz et al., 2001; O'Keefe and Pike, 2010) thereby limiting its perceived reproducability and effectively removing the assay from quality assurance protocols in food industry.

Oven Storage Test

The oven storage test requires a greater analysis time than the rapid methods discussed above, but is generally considered to be a more accurate means of elucidating the oxidative stability of fats and oils in an abbreviated timeframe. The oven storage test is carried out by holding a lipid sample of known volume in a forced-draft oven set to a temperature between slightly above ambient temperature and 80 °C, with the standard recommendation being 60 °C. Tests must be conducted in the dark, and the surface area to volume ratio must remain constant (O'Keefe and Pike, 2010). It has been demonstrated in previous studies that holding samples at temperatures of 60 °C in such a manner effectively accelerates lipid oxidation without greatly altering the mechanisms through which the lipids undergo oxidative decomposition (Frankel, 1993).

The oven storage test does not in itself provide a measure of oxidative stability; but rather, it accelerates the process of oxidation, such that storage stability tests can be completed in an abridged time frame. In order to determine the oxidative stability of lipids with the oven storage test, it is necessary to run parallel chemical methods designed to determine the present oxidative status of the lipids contained.

Measuring Current Oxidative Status

As discussed in our examination of autoxidation above, the initial compounds resulting from lipid oxidation can be categorized as being either "primary" or "secondary" products. Therefore, to gain a full understanding of the changes occurring in the oxidative status of lipids requires the assessment of both lipid oxidation product types. Some of the most commonplace assays for each category of lipid oxidation products will be discussed below.

Peroxide Value (1° products-no sensorial impact)

The peroxide value (PV) test is a titrimetric method used to determine the number of milliequivalents of peroxides present per kilogram of oil sample and is one of the most common tests used to assess the oxidative status of lipids (Shahidi and Zhong, 2005; O'Keefe and Pike, 2010). As aformentioned, the formation of hydroperoxides is a fundamental step in the process of autoxidation (see Eq. 3 above), and the rate of their accumulation can serve as an indication of the oxidative stability of lipids. Given the fact that hydroperoxides can be formed and decompose over the course of the oxidation of a lipid sample, the relative lack of hydroperoxides present at a moment in time may just as well suggest minimal oxidation of a lipid sample as it does thorough oxidative degradation. Thus, it is important to know the history of a lipid sample when running such an assay. When plotted over a period of time, the peroxide value of an oil will in most cases form an inverted "U shape," peaking at a middle point before declining due to hydroperoxide decomposition (Gharby et al., 2011). As such, PV is often used as a quality assurance indicator in food industry. Fresh refined oils and fats of high quality are expected to have peroxide values below 1; with values above 10 suggesting severe lipid oxidation has already taken place within the lipid sample (O'Keefe and Pike, 2010).

Conjugated Dienes and Trienes (1° products-no sensorial impact)

Once hydroperoxides are formed in the early stages of autoxidation, so too are conjugated double bonds or 'dienes' (Hamalainen et al., 2001). Conjugated dienes (in the case of oxidized PUFA with 2 double bonds) and trienes (3 double bonds) absorb UV light at 232nm and 270nm respectively, and are therefore easily quantified by a spectrophotometer (O'Keefe and Pike, 2010). This test is fast, requires no reagents, and can be helpful for monitoring lipid oxidation in

its early stages. It is important to note; however, that other conjugated compounds (*e.g.* carotenoids; refer to **Figure 2.10** for an example of the carotenoid β-carotene) can also absorb UV radiation at the discussed wavelengths, and thus, can interfere with the results of this test (Shahidi and Zhong, 2005).

p-Anisidine Value (2° products-sensorially active)

The *p*-Anisidine value test involves the measurement of the concentration of secondary oxidation products, α- and β- unsaturated aldehydes. The method relies upon the reaction between *p*-Anisidine and aldehydes producing a yellowish pigment under acidic conditions (Doleschall et al., 2002). The occurrence of this yellow pigment is then quantified *via* spectrophotometer at 350 nm (Gordon, 2001). The test is more sensitive to unsaturated aldehydes than to saturated ones, but studies have still shown the *p*-Anisidine value (*p*-AnV) of oils to have strong correlation with total volatile substances, as well as with sensory scores (List et al., 1974; Doleschall et al., 2002). The *p*-AnV is often incorporated into an equation with PV to form a TOTOX number (O'Keefe and Pike, 2010).

Thiobarbituric Acid Reactive Substances Test (2° products-sensorially active)

The thiobarbituric acid reactive substance test or 'TBARS test' (also often called TBARS), like the *p*-AnV, relies upon a reaction between an introduced reagent and secondary lipid oxidation products and can be measured by spectrophotometry (O'Keefe and Pike, 2010). The reagent, 2-thiobarbituric acid, interacts with malondialdehyde and malondialdehyde-type products to form a pink MA-TBA complex with an absorption maximum at 530-535 nm (Frankel, 1993; Antolovich et al., 2002). The TBARS test suffers from a lack of specificity and

sensitivity, but is still among the most commonly used methods to assess the oxidative state of lipids (de las Heras et al., 2003). In the case of bulk oils, the TBARS test is less popular than the use of the *p*-Anisidine Value test (Shahidi and Zhong, 2005).

Data Interpretation

The most informative assessment of the oxidative stability of lipids is realized through monitoring changes in the oxidative status of oils and fats over time. The resultant data of such analyses consists of a plot of different analytes incurred over time (refer to **Figure 2.12** for an example of such a plot). When assessing oxidation by more than one method, each will produce its own curve over time. In order to interpret the data of either one or more curves as a single quantitative indicator of oxidative stability, statistical techniques for consolidation and/or interpretation are required.

One strategy for data consolidation is the TOTOX (or total oxidation) Value, which is derived by an equation that incorporates results from both the *p*-Anisidine Value (*p*-AnV) assay and the Peroxide Value (PV) assays described above. The TOTOX equation appears below as equation (12) (Shahidi and Zhong, 2005):

Total Oxidation $$TOTOX = 2PV + p\text{-}AnV \quad (12)$$

Because the TOTOX equation incorporates both primary and secondary products of lipid oxidation, it typically rises continuously over time. Another calculation has been proposed in the literature for a revised TOTOX utilizing Thiobarbituric Acid Reactive Substances (TBARS) data instead of *p*-AnV values (Wanasundra and Shahidi, 1995). This revised TOTOX appears below as equation (13):

Total Oxidation $TOTOX_{TBARS} = 2PV + TBARS$ (13)

It should be mentioned; however, that because this value involves the combination of two indicators of oxidative status with different dimensions/units, it should not be considered as a finite relationship. It should also be noted that this value will still present a curve over time, so although it has reduced two curves into one, further interpretation is required in order to determine a single quantitative assessment of stability.

The curves of oxidation over time can be summarized and interpreted in a variety of ways. To date, there is no consensus standard regarding proper interpretative method by which to determine oxidative stability from oxidation plots. Imprecision and/or incomprehensiveness are problems that can occur with the implementation of any of these interpretative techniques, and variation in interpretative choice among scientists may be a contributing factor to observed inconsistencies of results and conclusions regarding oxidative stability. A summary of the different measures of curve interpretation that may be applied to oxidation studies are included below.

Area under the Curve

Area under the curve (AUC) is a calculation of integral calculus that determines the two-dimensional area under a plotted curve. This measurement is a very comprehensive single-term interpretation of a curve, as it is influenced by every single point of measured data. The width of high-magnitude regions within a curve is a powerful factor in the magnitude of a calculated AUC term, but the precise occurrence of a region upon the x-axis is not relevant. In the case of interpreting oxidation curves, this means that an earlier accumulation of oxidative products can

(meaningfully) increase an AUC score, but only if the earlier occurrence of these high magnitudes results in a longer duration of large quantity of products.

Induction Period

Induction period (or induction time) refers to the period of time before a notable increase in the accumulation of an analyte (Frankel, 1993). The most common example of use of this type of data interpretation is in the OSI test (Jebe et al., 1993) mentioned above. To ensure standardization, the induction period may be defined according to an ascribed maximum magnitude of tangential slope (Shahidi and Zhong, 2005). In other words, the induction period is considered to be over at the point in time that the slope of the curve begins to exceed a predetermined value.

Period Prior to Set Concentration

In lieu of an induction period mentioned above, a period of time prior to the accumulation of a set concentration can also be used. This period refers to the amount of time accrued until a cut-off point at a determined level of analyte concentration (Antolovich et al., 2002; Shahidi and Zhong, 2005). For instance, if an accelerated oven storage test is applied to a lipid (as discussed above) it can be calculated at what storage time/temperature relationship correlates with a pre-determined shelf-life period of that lipid at ambient temperature.

Concentration after Set Time Period

This is simply the evaluation of an analyte's observed concentration after a set time period. This measure should be uniform across all samples analyzed (Antolovich et al., 2002). Given the

propensity of primary products to degrade after formation, this method of data interpretation may be of more use in the evaluation of secondary products such as specific organic volatiles.

Rate of Oxidation

The rate of the oxidation of a lipid sample can also be expressed as the slope of the tangential line of the analyte's proliferation during a particular point in time (Princen et al., 1992). This specific point in time may be uniform across samples, or it may be assessed as uniform in relative orientation to other parameters within the plot (*e.g.* the rate might be assessed at the end of the induction period, regardless of the time at which that occurs). In some cases, such as with hydroperoxides, the evaluation of negative slopes occurring during the degradation of lipids may also be useful information. This rate of lipid oxidation is often expressed as concentration per unit time (Antolovich et al., 2002).

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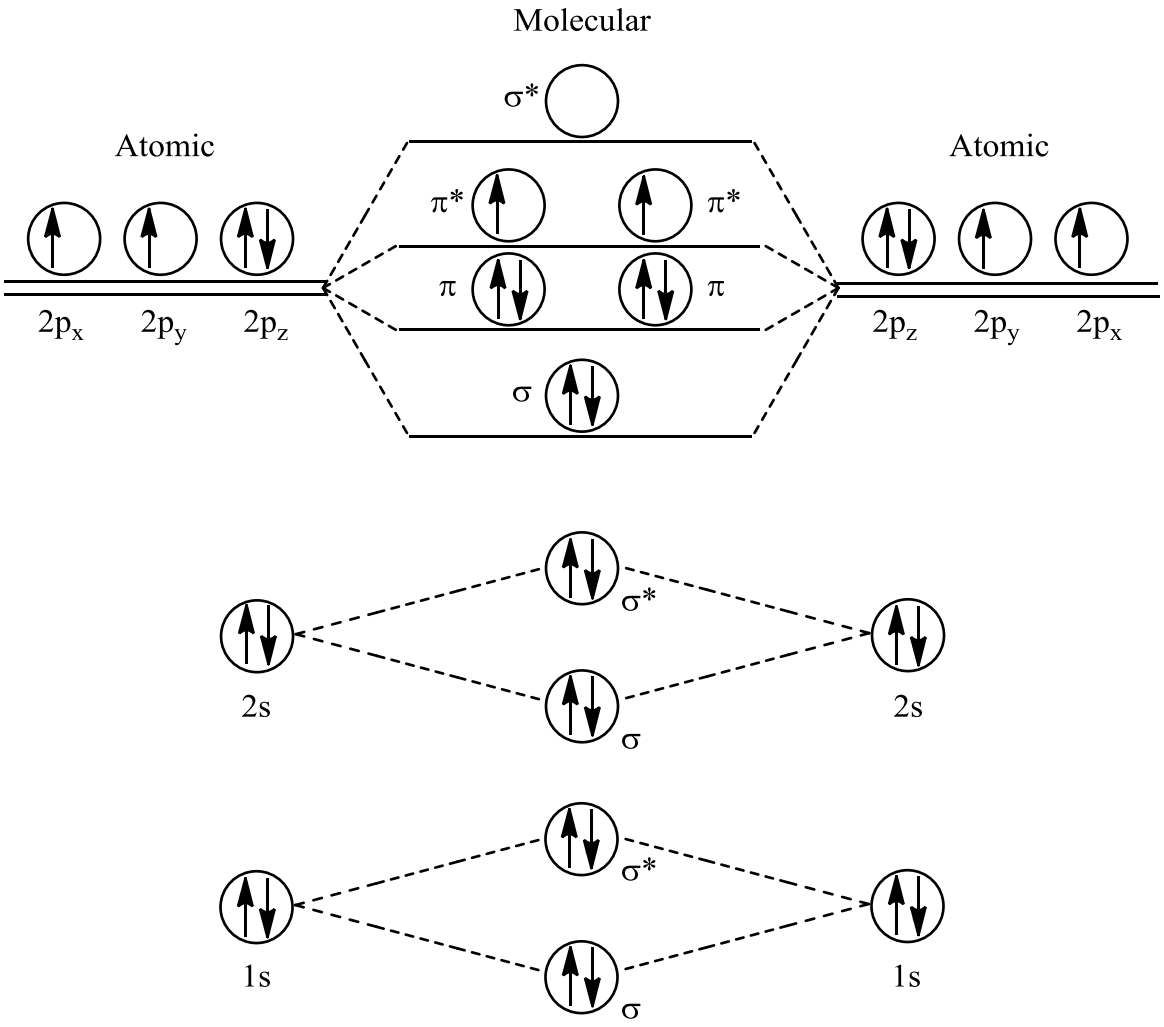


Figure 2.1 Molecular orbital of triplet oxygen (Min and Lee, 1999)

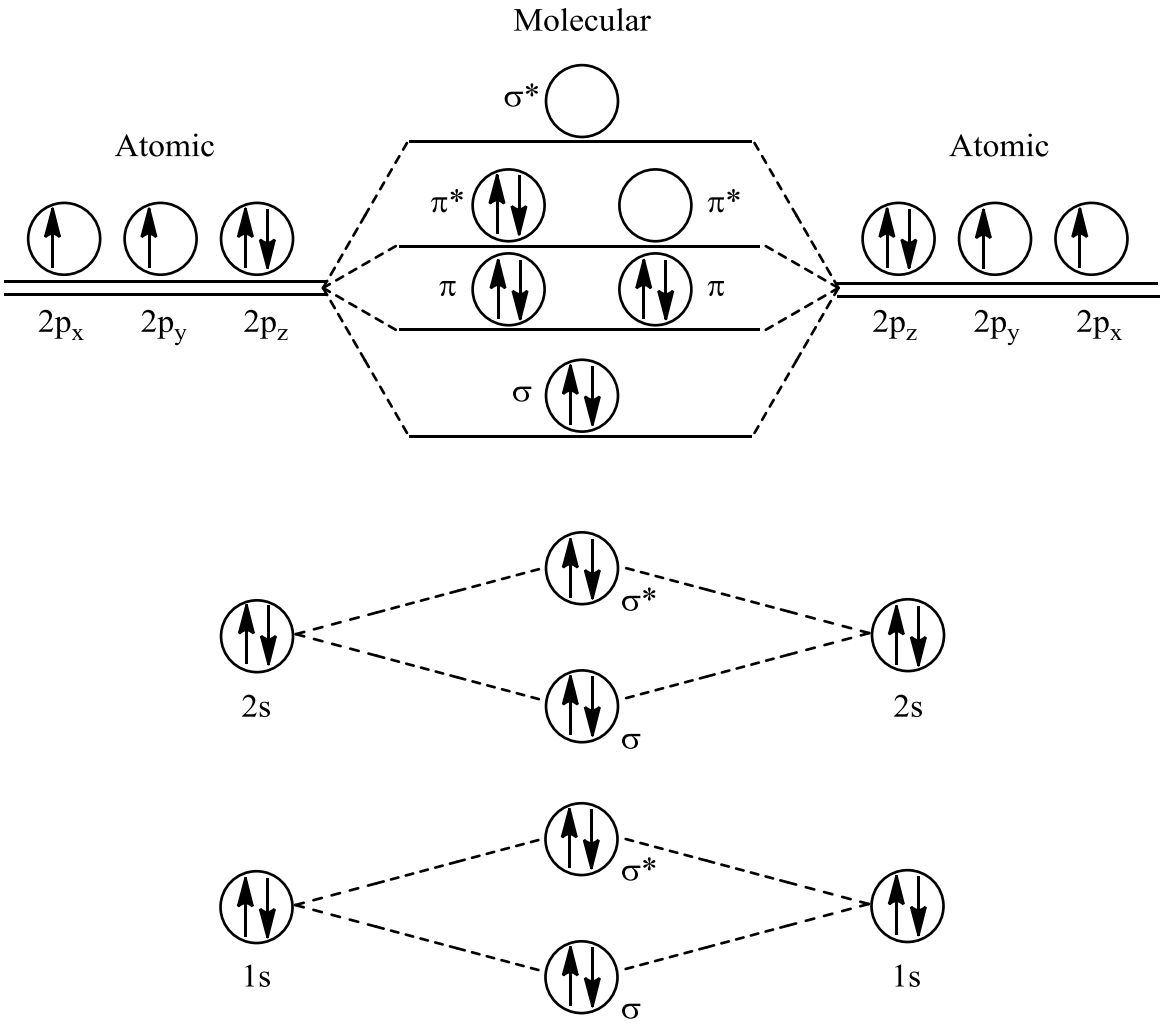


Figure 2.2 Molecular orbital of singlet oxygen (Min and Lee, 1999)

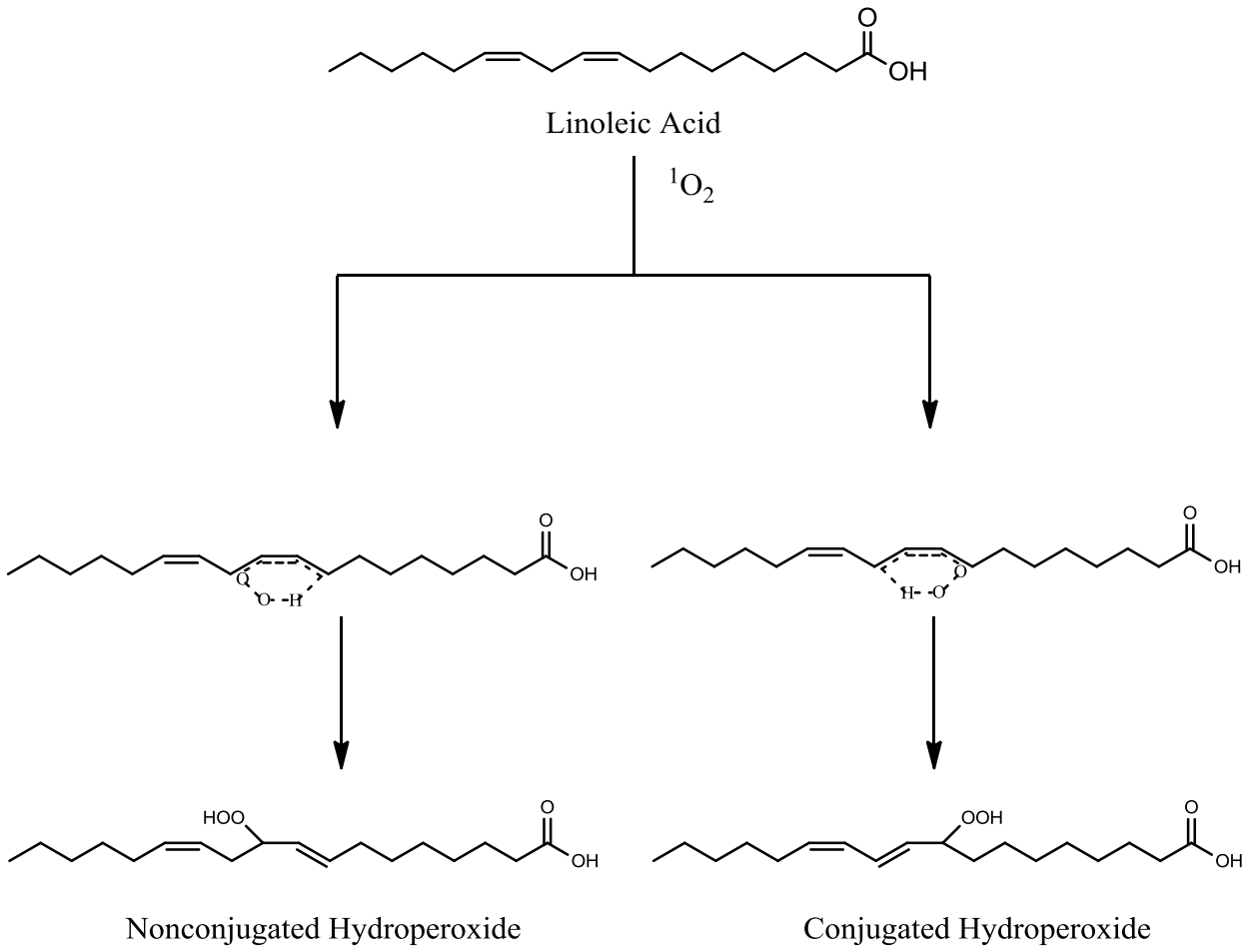


Figure 2.3 Reaction between linoleic acid and singlet oxygen (Choe and Min, 2009)

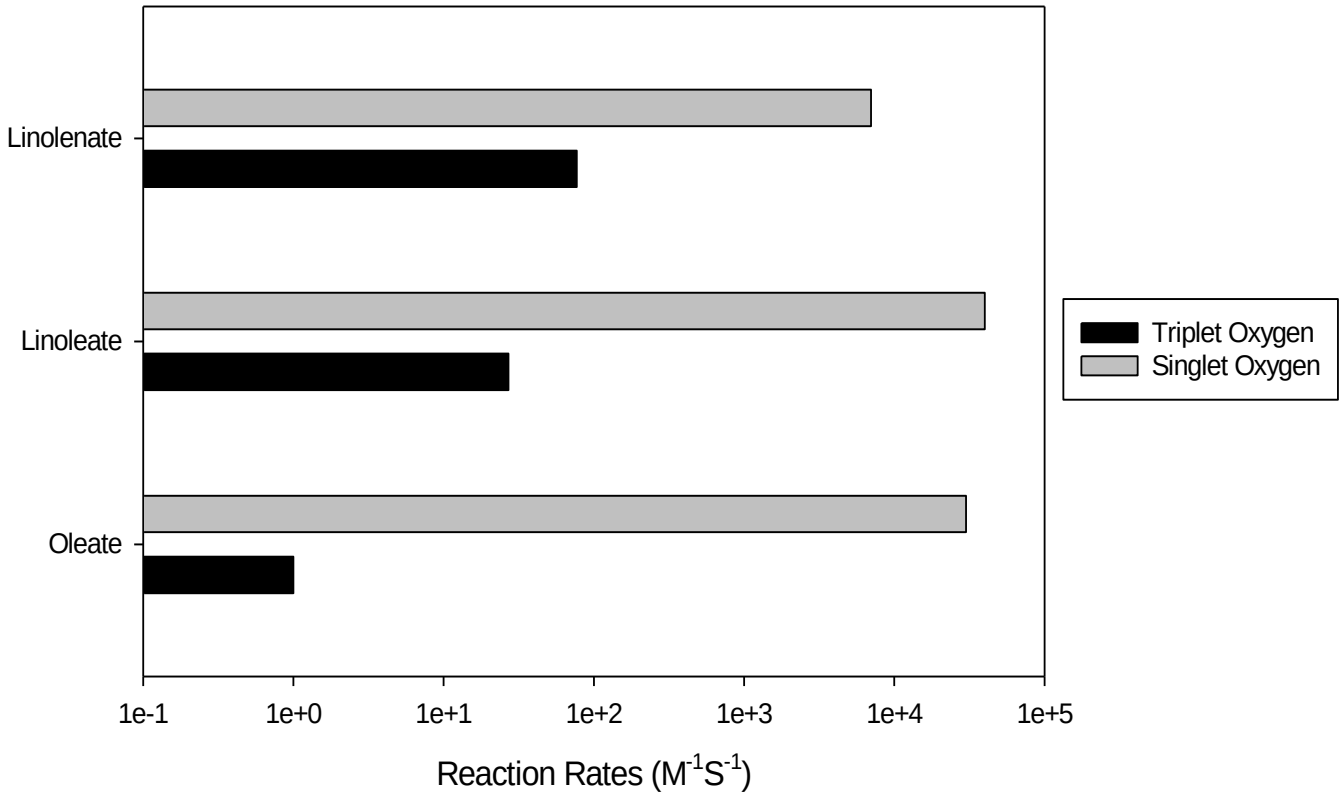


Figure 2.4 Relative reaction rates of singlet and triplet oxygen with different fatty acids (Gunstone, 1994)

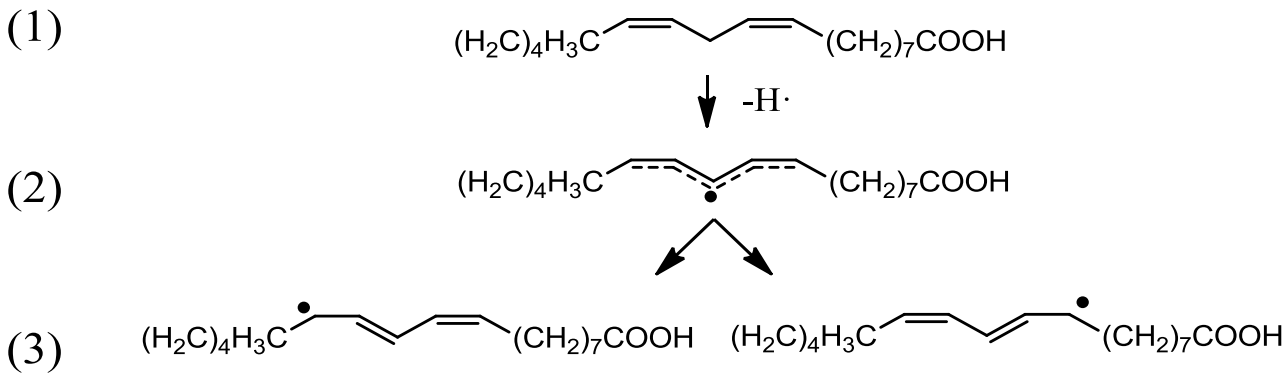


Figure 2.5 Formation of conjugated products through the autoxidation of linoleic acid (Choe and Min, 2006)

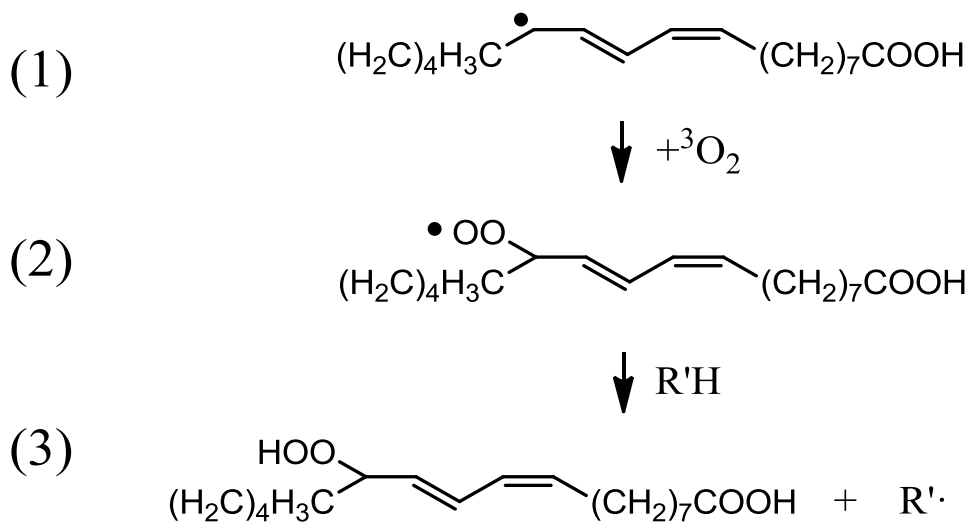


Figure 2.6 Formation of a hydroperoxide from the autoxidation of linoleic acid. This reaction proceeds *via* the formation of a conjugated radical. (Min and Boff, 2002)

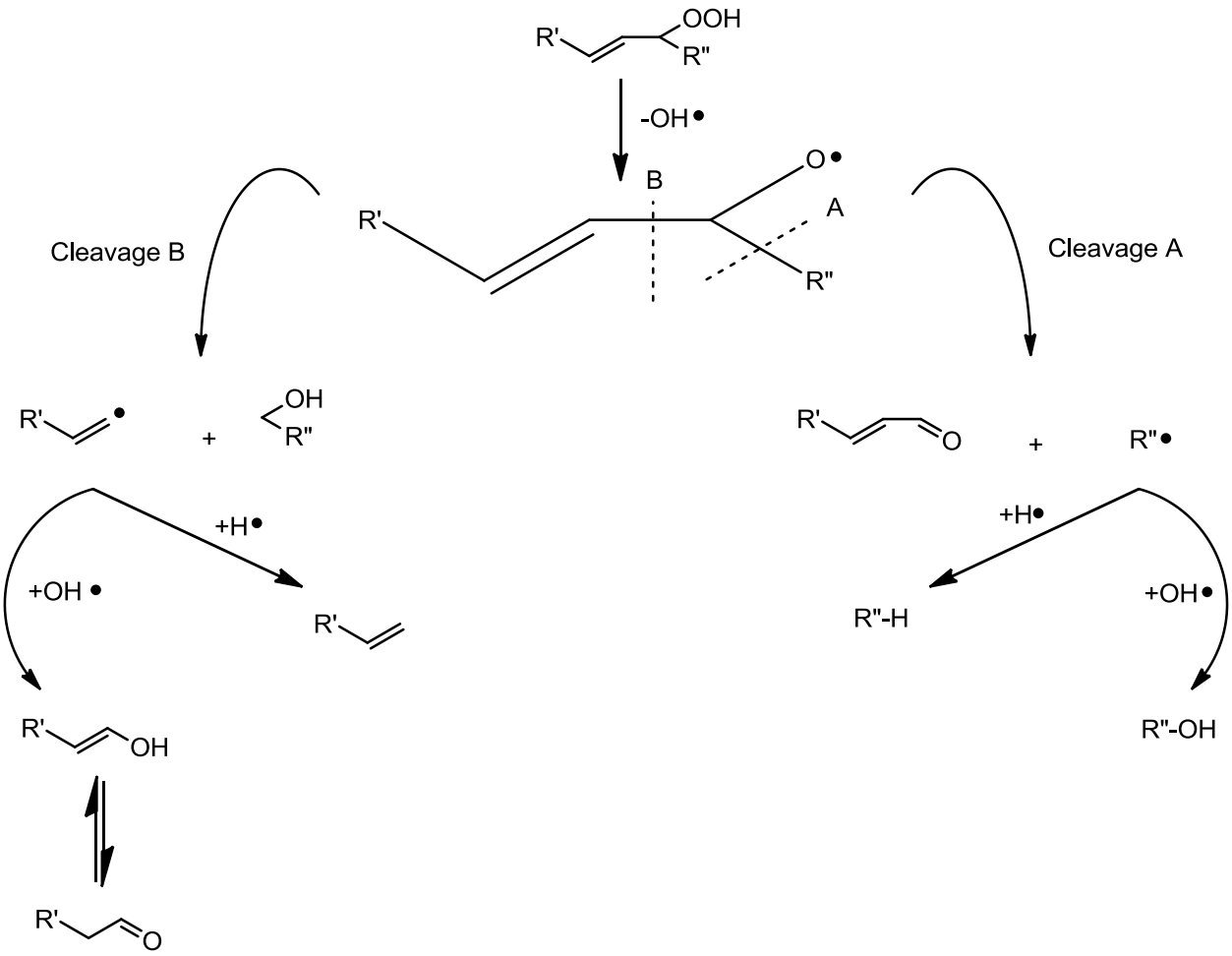


Figure 2.7 Potential pathways of the decomposition of hydroperoxides (Min and Boff, 2002)

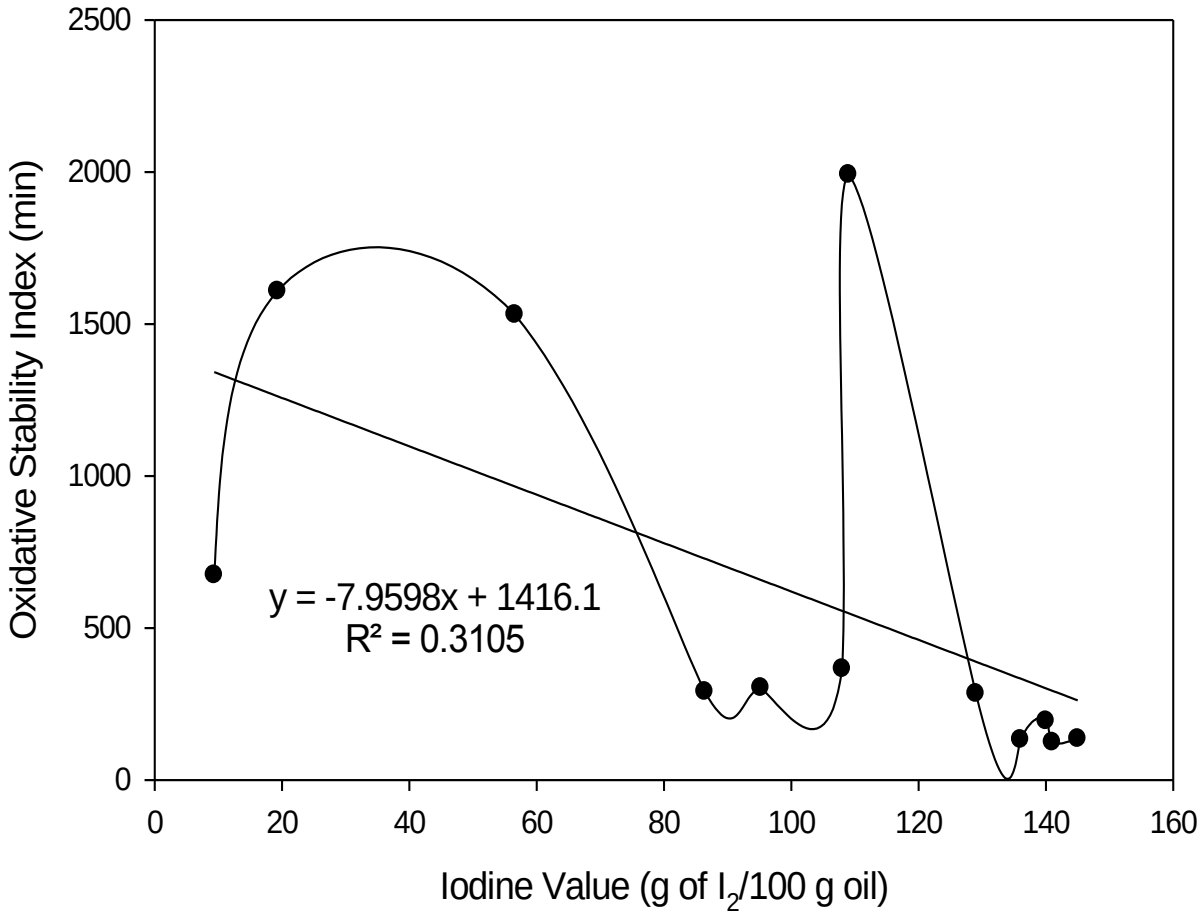


Figure 2.8 Oxidative stability index of edible oils tested as a function of their iodine values (Tan et al., 2002)

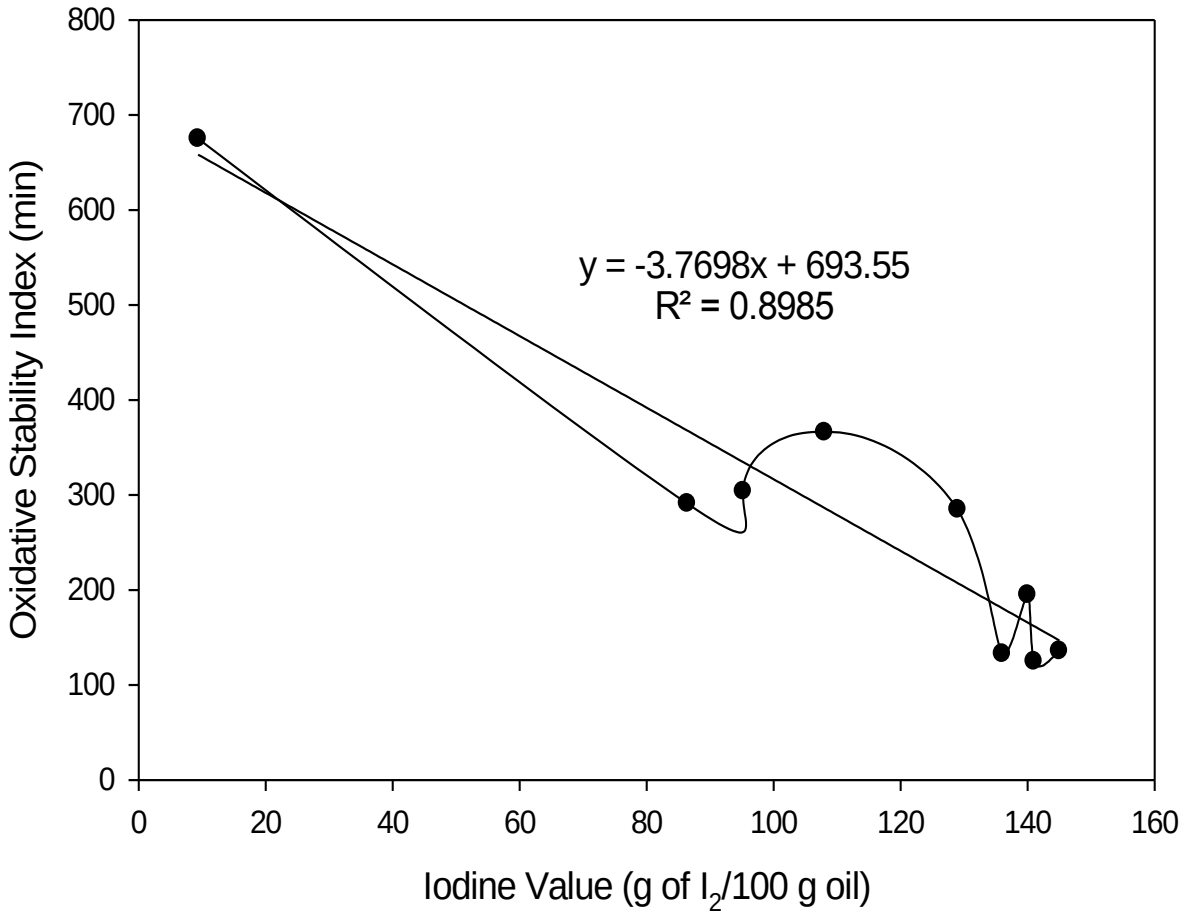
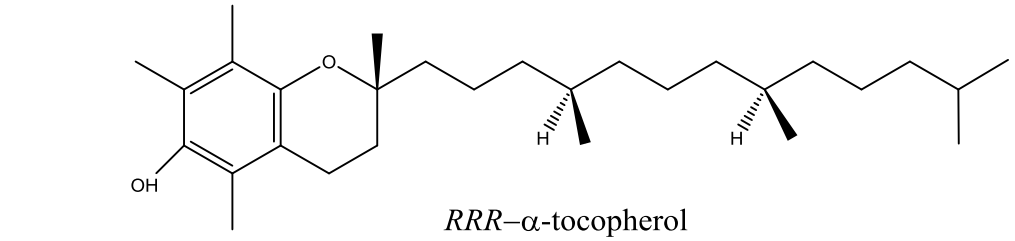
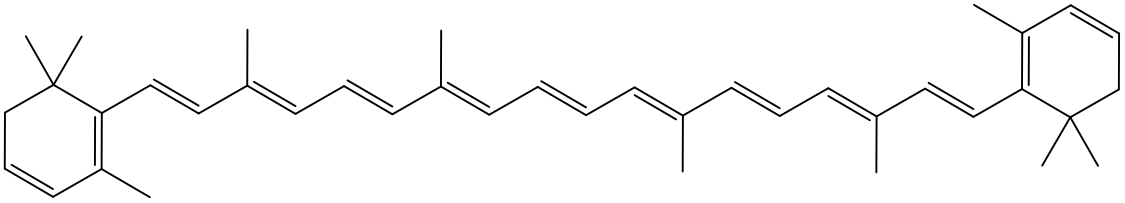


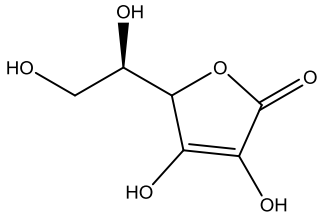
Figure 2.9 Oxidative stability index plotted as a function of iodine value of edible oils tested without the inclusion of outliers sesame oil, palm kernel oil, and palm oil olein fraction (Tan et al., 2002)



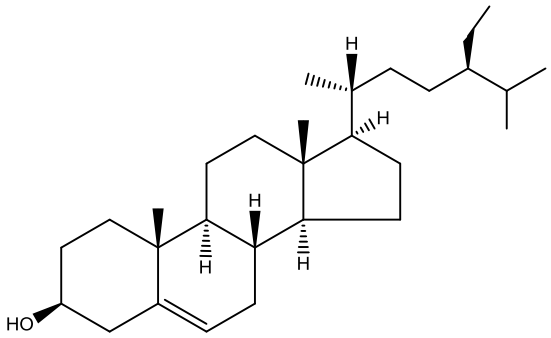
RRR–α-tocopherol



β-carotene



R-ascorbic acid



β-sitosterol

Figure 2.10 Chemical structures of some common natural antioxidants

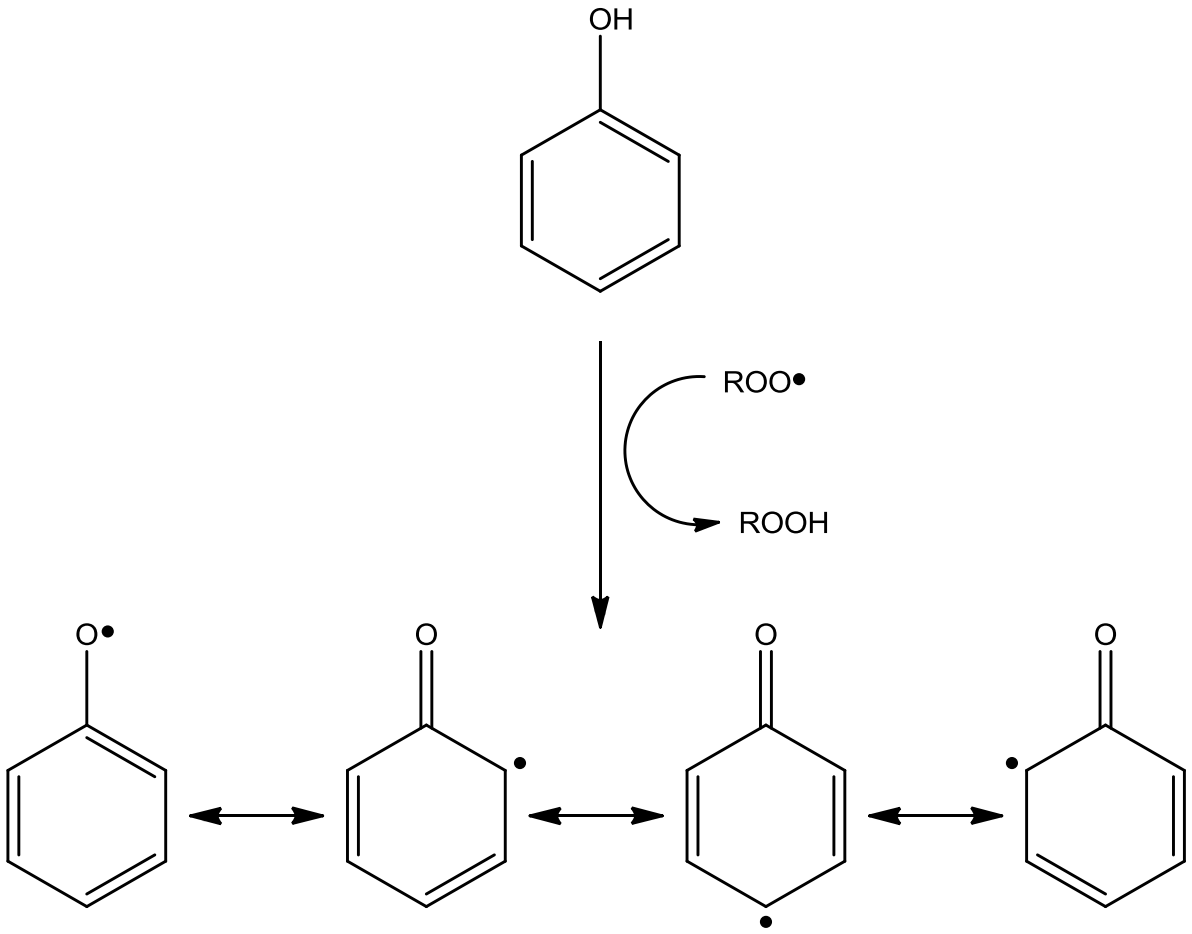


Figure 2.11 Mechanism of phenol antioxidant action: Conjugative resonance stabilization (Choe and Min, 2006)

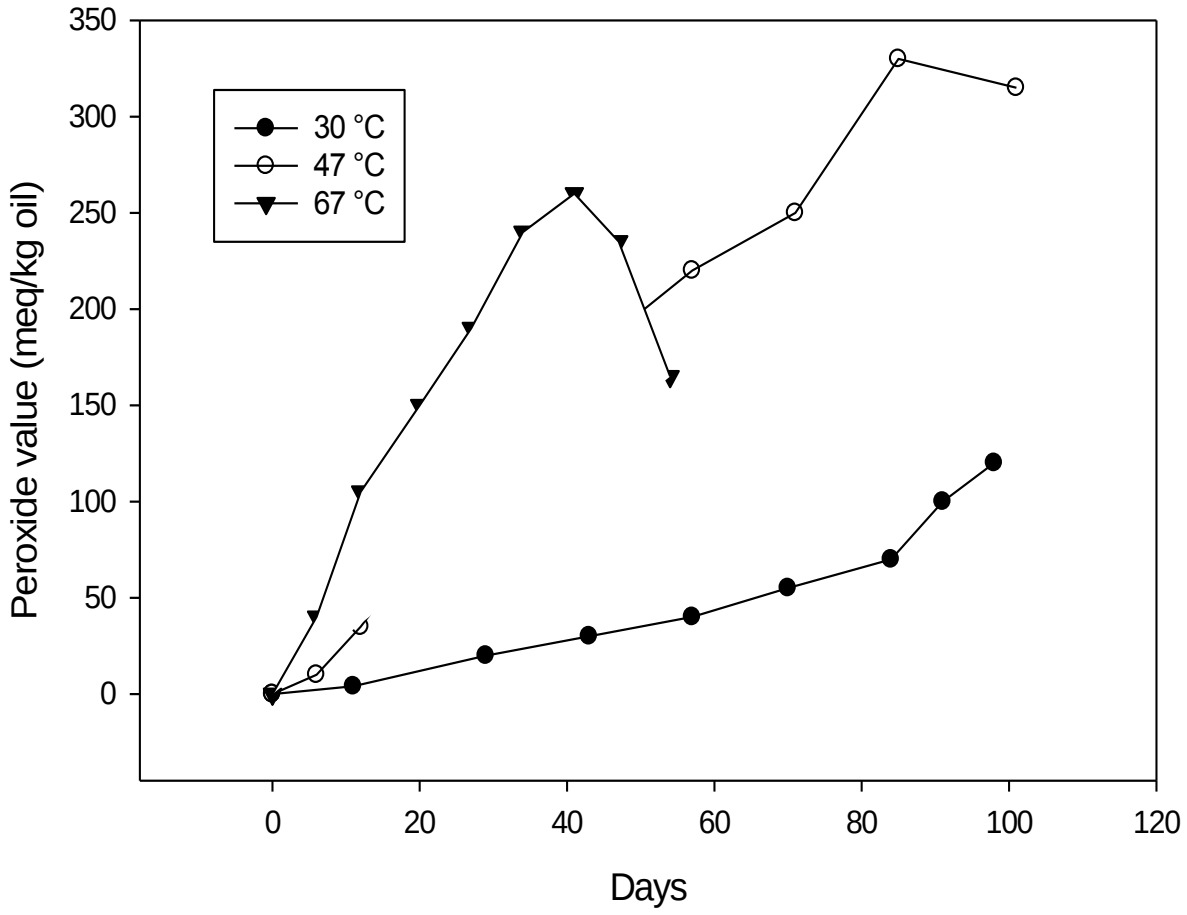


Figure 2.12 Peroxide values of sunflower oil at different storage temperatures (Crapiste et al., 1999)

Table 2.1 Free radical position on the fatty acid moiety following free-radical mediated autoxidation of various fatty acids (Frankel, 1985)

Fatty acid	Location of radical	Relative amount
Oleic acid	C8	~27%
	C9	~23%
	C10	~23%
	C11	~27%
Linoleic acid	C9	~50%
	C13	~50%
Linolenic acid	C9	~32%
	C12	~11%
	C13	~12%
	C16	~32%

Table 2.2 Typical decomposition products of hydroperoxides following β-scission (Chaiyasit et al., 2007)

Chain cleavage oxidation products	**Di- and polymerization products:**	**Rearrangement products**
Alkanals, 2-Alkenals, Alkanes, 2,4-Alkadienals, Alkatrienals, α-Hydroxyaldehydes, Ketones, Malonaldehyde	Dimers and polymers linked ether, carbon, and/or peroxy bridges.	Hydroxy acids, Keto acids.

Table 2.3 Sensory threshold values for some common oxidation products (Frankel, 1985)

Compounds	**Threshold (ppm)**
Vinyl ketones	0.00002-0.007
trans, cis-alkadienals	0.002-0.006
Isolated *cis*-alkenals	0.0003-0.1
Isolated alkadienals	0.002-0.3
trans, trans-2,4-Alkadienals	0.04-0.3
Alkanals	0.04-1.0
2-Alkenals	0.04-2.5
1-Alkenes	0.02-9
Vinyl alcohols	0.5-3
Substituted furans	2-27
Hydrocarbons	90-2150

Table 2.4 Sensory descriptors for some common oxidation products (Min and Bradley, 1992; Villiere et al., 2007)

Sensory Descriptor	**Compounds**
Cardboard	*trans,trans*-2,6-Nonadienal
Cut-grass, grassy	Hexanal, *trans*-2-Hexenal, Nona-2,6-dienal
Deep-fried	*trans,trans*-2,4-Decadienal
Fishy	*trans,cis,trans*-2,4,7-Decatrienol, Oct-1-en-3-one
Fresh oil, oily	Aldehydes
Mushroom	1-Octen-3-ol
Painty	Pent-2-enal, Aldehydes
Wood bug	*trans*-2-Octenal

CHAPTER 3

DEVELOPMENT AND DERIVATION OF SINGLE QUANTITATIVE TERM FOR THE COMPREHENSIVE DESCRIPTION OF LIPID OXIDATIVE STABILITY

INTRODUCTION

Lipid oxidation is one of the central economic concerns to both the edible oil and food & beverage industry. The oxidative deterioration of lipids in food products can affect food safety, nutrition, texture, color, as well as result in the release of aromatic volatile compounds responsible for undesirable or "rancid" flavors associated with poor food quality (Gray, 1978; Ajuyah et al., 1993; Morales et al., 1997). Lipid oxidation is in many cases the limiting factor in the shelf life of food products, and thus, is one of the key elements that require consideration in a product's design, formulation, processing, packaging, and storage (Chaiyasit et al., 2007).

Many contributing factors to lipid oxidation have been cited including: fatty acid composition (particularly the degree of unsaturation), storage temperature, processing methods, and the concentrations of oxygen, free fatty acids, pro-oxidants, and antioxidants. However, the observed effects of these factors in scientific study have been highly inconsistent – which has meant that means to accurately predict and/or control lipid oxidation have proved elusive (Lea and Hawke, 1951; Min and Boff, 2002). One possible explanation for the inconsistencies in scientific literature is that the interaction of these factors (as well as matrix effects) has not been well understood on a quantitative level (Choe and Min, 2006; Chaiyasit et al., 2007). To this point, even when focusing only upon a single variable of lipid oxidation within a single lipid source, studies have often failed to find consistent relationships. For example, higher antioxidant

concentrations in bulk oils do not necessarily directly correlate with higher lipid stability (Satue et al., 1995; Baldioli et al., 1996). Results such as these suggest there are interactions with other factors in ways not yet fully understood.

An investigation of published literature studies reveals that another source of confusion and irregularity may lie in the inconsistent assessments and definitions of oxidative stability. Currently, the term 'oxidative stability' is a fairly nonspecific term that refers to the exhibited resistance of a lipid or lipid-containing food product to undergo oxidation (Guillén and Cabo, 2002; Velasco and Dobarganes, 2002). The "level of resistance" is a concept without quantitative definition. As such, oxidative stability may be determined according to a multitude of methodologies, and also according to many numerical interpretations. Traditional assessments of stability include repeated assessments of oxidation products over elapsed time, which result in plotted curves of products over time (see **Figure 3.1** for an example of such a curve**)**. Such curves may then be interpreted in many ways – including the following common techniques:

- Area Under Curve
- Maximum Value
- Time of Maximum Value
- Time of 20% Maximum Value
- Integrated Area prior to 20% Maximum Value
- Maximum Value/Time of Maximum Value
- Slope of Tangent Line During Growth Phase

These differing techniques of interpretation can lead to dramatically different conclusions. **Figure 3.2** depicts this phenomenon. In this example (taken from the results of this study), two

different samples produce peroxides (indicators of primary oxidation) over time in a manner that does not clearly demonstrate one sample's stability being greater than the other. The numbers in the figure show multiple numerical interpretations that can be applied to these curves, and the numbers in bold indicate evidence of the sample's greater stability according to that numerical method. In this example, we are comparing only two samples, doing so only in a binary manner (i.e. qualitatively assessing which sample is more stable, rather than quantifying the degree of differences), and utilizing only one methodology from which oxidation data was collected simultaneously and identically. Still, the conclusion regarding the comparative stability of these two samples is demonstrated here to be entirely dependent upon the choice of numerical interpretation. If, for example, two studies were attempting to discern the effect of higher fatty acid concentrations within oil samples upon stability, then the conclusions could be exactly opposite one another even if they produced the exact same data but used different numerical interpretations. It is easy to envision how this effect could be compounded greatly when the comparisons are between large numbers of samples, quantitative, and span multiple assays, labs, experimenters, study designs, etc. This study attempts to address this problem by producing a comprehensive meaningful quantitative definition of oxidative stability. It is hoped that future scientists will consider acquiring the value determined here so that scientific literature on the subject may advance with less inconsistency.

Any attempt to devise a good means by which to quantify oxidative stability will suffer somewhat from a lack of means of validation. In other words, there is no "true" known stability that the output of quantitative techniques can be compared to. However, it is still possible to produce a set of criteria that a good numerical technique should meet. For this study, those criteria are as follows:

1. Numerical interpretation should show good consistency across different assays commonly used to assess oxidation.
2. Magnitude of term should ideally be influenced by both the magnitude of observed oxidation products and the rate of their proliferation.
3. Term should include consideration of both primary oxidation products and secondary oxidation products.
4. Term should avoid placing excessive emphasis on the specialities of individual assays.
5. Ideally, the quantified term should be able to be assessed (or at least approximated) without too much time and effort.

This study includes a large-scale comprehensive assessment of the oxidation of 50 commercial-use oils and fats. These samples were assessed by four common assays (two assessing primary oxidation and two assessing secondary oxidation) repeatedly throughout two months of accelerated storage. The resultant data was then the subject of numerous statistical summation techniques, and the criteria above were used as a means of identifying good quantitative summations that could meaningfully numerically describe a sample's oxidative stability.

MATERIALS AND METHODS

Samples: Selection, Handling, and Screening

50 commercially available fat & oil samples were provided by the Nestlé Research Center/NESTEC Ltd. (Lausanne, Switzerland). Their fatty acid composition was established by gas chromatographic analysis. **Table 3.1** lists these edible oils in order of ascending

unsaturation. This table also includes the samples' Calculated Iodine Values (CIV). All samples were verified by HPLC analysis to be free of added synthetic antioxidants, according to AOAC official method 983.15.

Experimental Design

Aliquots of all lipid samples were placed in accelerated storage conditions and assessed over 63 days by four distinct validated methods of analysis, including the determination of both primary and secondary products of lipid oxidation. Primary products of lipid oxidation were monitored by the peroxide value test and an assessment of conjugated dienes and trienes. Secondary oxidation products such as malondialdehyde, 2-alkenals, and others were assessed by the thiobarbituric acid reactive substances test and the *p*-anisidine value assay. All assays were performed upon samples stored in duplicate and the mean values were reported.

Oven Storage Test

Accelerated storage testing was carried out according to oven storage test protocol AOCS Cg 5-97. Lipid samples were each individually dispensed into 110 separate aliquots of 4 mL and held in identical 20 mL amber glass storage vials. Each vial was then covered and placed in thermostatically controlled gravity convection ovens (Fisherbrand™ Isotemp Incubators) at a constant temperature of 60 °C (AOCS, 1998). Temperatures were dual-monitored by Fisherbrand™ Traceable™ Snap-in Module Thermometer with Probes and Fisherbrand™ Red-Spirit™ No-Roll Laboratory Thermometers. All samples were analyzed by all analytical assays after 0, 1, 3, 7, 10, 14, 18, 23, 29, 36, 43, 50, and 57 days. The peroxide value and conjugated fatty acids assays were also performed following 63 days of storage. Each aliquot was used for

assessment only once and then discarded, ensuring that each aliquot remained entirely undisturbed and unaltered prior to its assessment. Duplicate storage was achieved by the storage of aliquots under identical conditions within separate vials.

Peroxide Value

The peroxide value (PV) test is a titrimetric method used to determine the number of milliequivalents of peroxides present per kilogram of oil sample and is one of the most common tests used to assess the oxidative status of lipids (Shahidi and Zhong, 2005; O'Keefe and Pike, 2010). The concentration of hydroperoxides within samples was quantified according to the AOCS official method Cd 8b-90 (AOCS, 1998). Results are reported as mEq O_2/kg according to the following equation (1):

PV Equation

$$\text{Peroxide Value} = ((S - B) \times N \times 1000)/m \quad (1)$$

(where S, B, N, and m are equivalent to the sample titration volume, in ml, blank titration volume, in ml, normality of sodium thiosulphate solution, and mass of the test portion, respectively).

Conjugated Dienes and Trienes

As hydroperoxides are produced within lipid samples, conjugated double bonds will commonly be present within these compounds. Conjugated dienes (CD; 2 conjugated double bonds) and trienes (CT; 3 conjugated double bonds) absorb UV light at 232nm and 268nm respectively, and can therefore be quantified by a spectrophotometer (O'Keefe and Pike, 2010).

The concentrations of conjugated dienes and trienes within the samples were determined in accordance with IUPAC Official method 2.505 (IUPAC, 1987), utilizing a Agilent 8453 UV-visible Spectroscopy System and VWR International 10mm Quartz Spectrophotometer Ultraviolet Rectangular Cells. The results were reported as sample extinction coefficients E1% according to the following equation (2):

CDT Equation $$E1\% = A_\lambda/(c_L \times l) \quad (2)$$

(where E, A_λ, c_L, and l represent the extinction value, the absorbance measured at either 232 nm (for CDs) or 268 nm (for CTs), the concentration of the lipid solution in g/100 ml, and the path length of the quartz cuvette in cm, respectively).

At times, the reporting and interpretation of the conjugated dienes and trienes assay is achieved by a summation of observed conjugation with a sample. For this purpose, the extinction coefficients for conjugated dienes were summed with the extinction coefficients for conjugated trienes. This value is reported as the conjugated dienes and trienes value (CDT).

Thiobarbituric Acid Reactive Substances Test

The thiobarbituric acid reactive substance test or 'TBARS test' relies upon a reaction between an introduced reagent and secondary lipid oxidation products and can be measured by spectrophotometry (O'Keefe and Pike, 2010). 2-Thiobarbituric acid interacts with malondialdehyde and malondialdehyde-type products to form a pink MA-TBA complex with an absorption maximum at 530-535 nm (Frankel, 1993; Antolovich et al., 2002). The TBARS test was carried out according to AOCS official method Cd, 19-90 (AOCS, 1998), utilizing a Agilent

8453 UV-visible Spectroscopy System and VWR International 10mm Quartz Spectrophotometer Ultraviolet Rectangular Cells. The resulting data is reported as a TBARS value according to the following equation (3):

TBARS Equation $$TBARS_{Value} = (50 \times (A - B))/m \quad (3)$$

(where A, B, and m are equivalent to the absorbance of the test solution, absorbance of the reagent blank, and mass of the test portion in grams, respectively).

p-Anisidine Value Assay

The *p*-anisidine value (or *p*-AnV) assay involves the measurement of the concentration of secondary oxidation products, α- and β- unsaturated aldehydes. The method relies upon the reaction between *p*-anisidine and aldehydes producing a yellowish pigment under acidic conditions (Doleschall et al., 2002). The occurrence of this yellow pigment is then quantified *via* spectrophotometer at 350 nm (Gordon, 2001). The *p*-AnV assay was carried out on lipid samples according to AOCS official method Cd 18-90 (AOCS, 1998), utilizing a Agilent 8453 UV-visible Spectroscopy System and VWR International 10mm Quartz Spectrophotometer Ultraviolet Rectangular Cells. The resulting data is reported as a *p*-AnV according to the following equation (4):

***p*-AnV Equation** $$p\text{-AnV} = (25 \times (1.2As - Ab))/m \quad (4)$$

(where As, Ab, and m are equivalent to the absorbance of the fat solution after reaction with the *p*-anisidine reagent, absorbance of the fat solution, and sample mass in grams, respectively).

Calculated Iodine Value

To determine summations of total unsaturation within the samples, Calculated Iodine Values (CIV) were evaluated according to AOCS official method Cd 1c-85 (AOCS, 1998). This calculation sums the proportional content of all unsaturated fatty acids, each multiplied by a factor accounting for both the number of double bonds within the compound as well as its molecular weight. The results are reported as g iodine/100 g oil in reference to the traditional iodine value assay of which these results are intended to closely approximate.

Assessed Oxidation by Numerical Interpretations

The following numerical interpretations were performed on the oxidation data for each assay:

- Area Under Curve
- Maximum Value
- Time of Maximum Value
- Time of 20% Maximum Value
- Integrated Area prior to 20% Maximum Value
- Maximum Value/Time of Maximum Value
- Slope of Tangent Line During Growth Phase

In most cases, the means by which to acquire these numbers is self-explanatory. Those regarding integrated areas and slopes of tangent lines are explained below.

Area under the Curve

Area under the Curve (AUC) values and Integrated Area prior to 20% Maximum values were computed using integral calculus computations within Sigmaplot 12.0 (Systat Software, San Jose, CA).

Slope of Tangent Line during Growth Phase

The "growth phase" was considered to be the time of marked upward proliferation of oxidation products, and was determined by visual inspection of the curves. The slope of the tangent line for that time period was calculated by linear regression.

Consistency of Numerical Interpretation across Assays (Criteria #1)

To determine the consistency of each numerical interpretation across assays (as a means to address criteria #1 above), a sequence of rankings and standard deviations were employed. For each numerical interpretation technique, the samples were ranked (i.e. 1 through 50) within each assay (PV, CDT, TBARS, and *p*-AnV) according to the stability exhibited by the sample. From this, each numerical interpretation produced four discrete numerical values for each sample (the ranked value of each of the four assays). The standard deviation of these four scores was then computed for each sample, and the standard deviation scores of the 50 samples were averaged to produce an "Average Deviation Across Assays" (ADAA) score. Practically speaking, the ADAA score shows how much the ranking of a sample varies among various comparative approaches. This value is without units and is used strictly for comparison purposes. This value is inherently standardized to comparable magnitudes across numerical interpretation approaches due to the nature of ranked values.

Standardized Values (Criterion #3 and #4)

As a means to address the criterion #3 and #4 listed in the introduction, a standardization step is utilized in this study to provide equal weight to all assays in a consolidated value. The standardization step was accomplished by multiplying the values of CDT, *p*-AnV, and TBARS by a set of constant coefficients so that they are on a scale of comparable magnitude to that of PV. Once standardized, the AUC values were calculated again (this time on a comparable scale across assays), and subsequently averaged within each sample and across all assays.

Linear Regression (Criteria #5)

In order to lessen the data collection required to achieve a good summarized score of stability, multiple linear regression was performed upon the raw data points of the assays to find a good approximation of our final determined assessment of oxidative stability.

RESULTS AND DISCUSSION

Assessed Oxidation by Numerical Interpretations

Tables 3.2 – 3.8 show the assessed oxidation of the 50 samples according to area under curve, maximum value, time of maximum value, time of 20% maximum value, integrated area prior to 20% maximum value, maximum value/time of maximum value, and slope of tangent line during growth phase, respectively. For each numerical interpretation technique, the interpretations are shown for each assay independently. These values show general trends of greater stability with greater saturation, which is in accordance with expectations.

Consistency of Numerical Interpretation across Assays (Criteria #1)

Table 3.9 shows the "Average Deviation Across Assays" (ADAA) score of each of the seven numerical interpretation techniques. The numerical interpretation technique that showed the greatest consistency between assays was the AUC value, with an ADAA of 3.98. Maximum value performed nearly as consistently, with an ADAA of 4.26. The time of maximum value was the least consistent among the numerical interpretations assessed, with an ADAA of 10.4.

From these results, we consider the AUC to be the least vulnerable to differing conclusions due to differing assays. The AUC has the additional benefit of being one of only several interpretation techniques for which the magnitude is influenced both by magnitude of products and accumulation rate, which addresses criteria #2 above. For these reasons, AUC serves as the basis for the development of our quantitative definition of stability.

Standardized Values (Criterion #3 and #4)

To create a term which accounts for both primary oxidation products and secondary oxidation products equally, and also neither overemphasizes nor underemphasizes the individual information from each assay, a standardized summation of AUC (St. Sum AUC) was produced. According to the relative magnitudes of the average AUC values of the four assays (see Appendix A), the equation for this value was derived as follows (5):

$$\text{St. Sum AUC} = [PV_{AUC} + (6.31)CDT_{AUC} + (2.60)TBARS_{AUC} + (2.87)p\text{-}AnV_{AUC}]/4 \quad (5)$$

This derived term is proposed to be a meaningful and comprehensive quantitative summary of an oil or fat's oxidative stability, effectively addressing the first three criteria for such a value

discussed in the introduction. The relationship between St. Sum AUC and oxidative stability is an inverse one (i.e. higher St. Sum AUC values should be interpreted as signifying lesser oxidative stability). The St. Sum AUC values of the 50 samples are shown in **Table 3.10**. Although this term could be creatively adapted to a variety of study designs, any determination of this value intended for the comparison to that of other studies would require an adherence to the study design as outlined with minimal modification.

Approximation of Standardized Sum of AUC by Linear Regression (Criteria #5)

A potential problem with the proposed derived term of St. Sum AUC is the extensive laboratory work required to acquire such a value. To address this problem, multiple linear regression was performed upon the data points to find a good approximation of the St. Sum AUC (~St. Sum AUC) that require a great deal less laboratory work. The result is as follows (6):

$$\sim\text{St. Sum AUC} = 150 + (209)CDT_{\text{Day 14}} + (82.2)CDT_{\text{Day 43}} + (32.7)TBARS_{\text{Day 36}} \quad (6)$$

This approximate term has a very strong correlation with the more labor-intensive term ($R^2_{adj.}$ = 95.2%), and requires the acquisition of only three data points. When the attainment of the complete St. Sum AUC value is considered either impractical or infeasible, this value can be attained instead and should still be meaningfully comparable to St. Sum AUC values.

CONCLUSIONS

In a comparison of seven commonly used numerical interpretations of lipid oxidation curves, it was determined that Area Under the Curve was the least vulnerable to variations

between methods of assessing oxidation products. AUC is therefore suggested to be a good basis for a new quantitative definition of oxidative stability. The most comprehensive definition of exhibited oxidation was determined to be a Standardize Summation of AUC value which equally incorporates information from four common assays. A very good approximation of this comprehensive measurement can be attained with only three data points acquired over the course of 43 days of accelerated storage. It is suggested that future studies regarding oxidative stability within oils and fats consider acquiring and reporting this value, so that meaningful quantitative comparisons can be made between studies. If scientists were to consistently consider this term a meaningful quantitative definition of stability, the frequency of contradictory conclusions within literature may be minimized.

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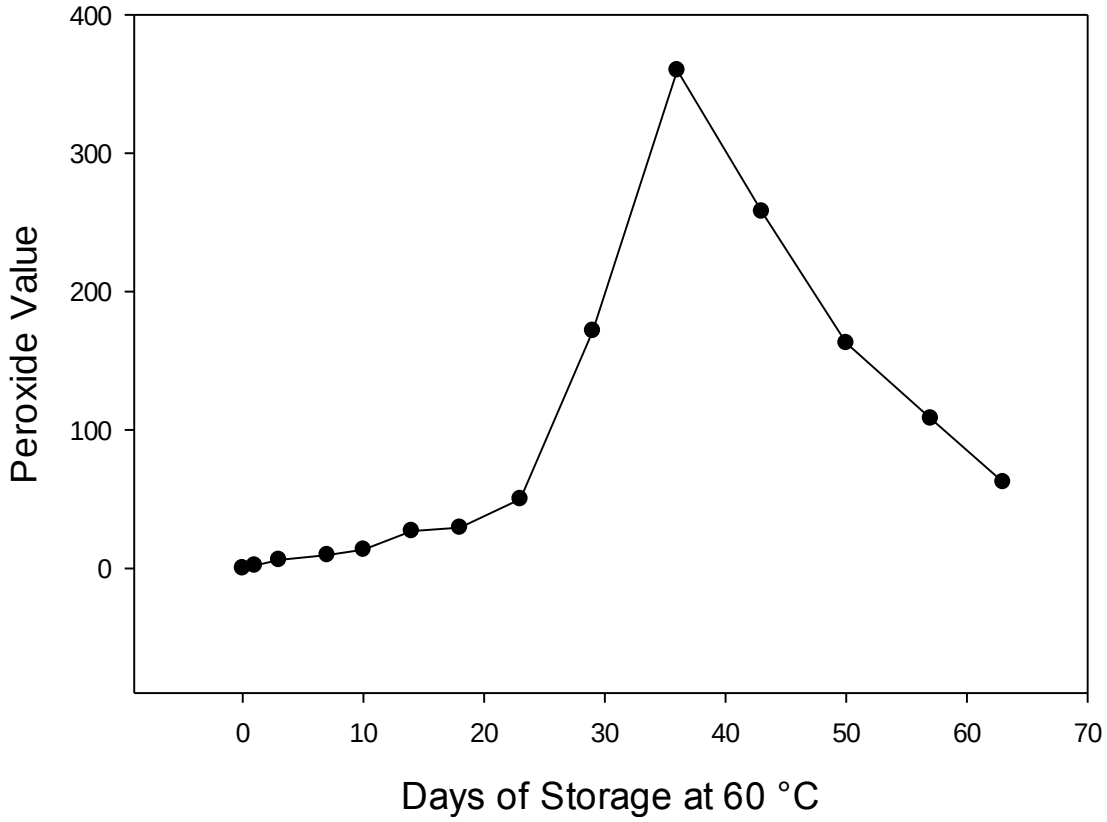
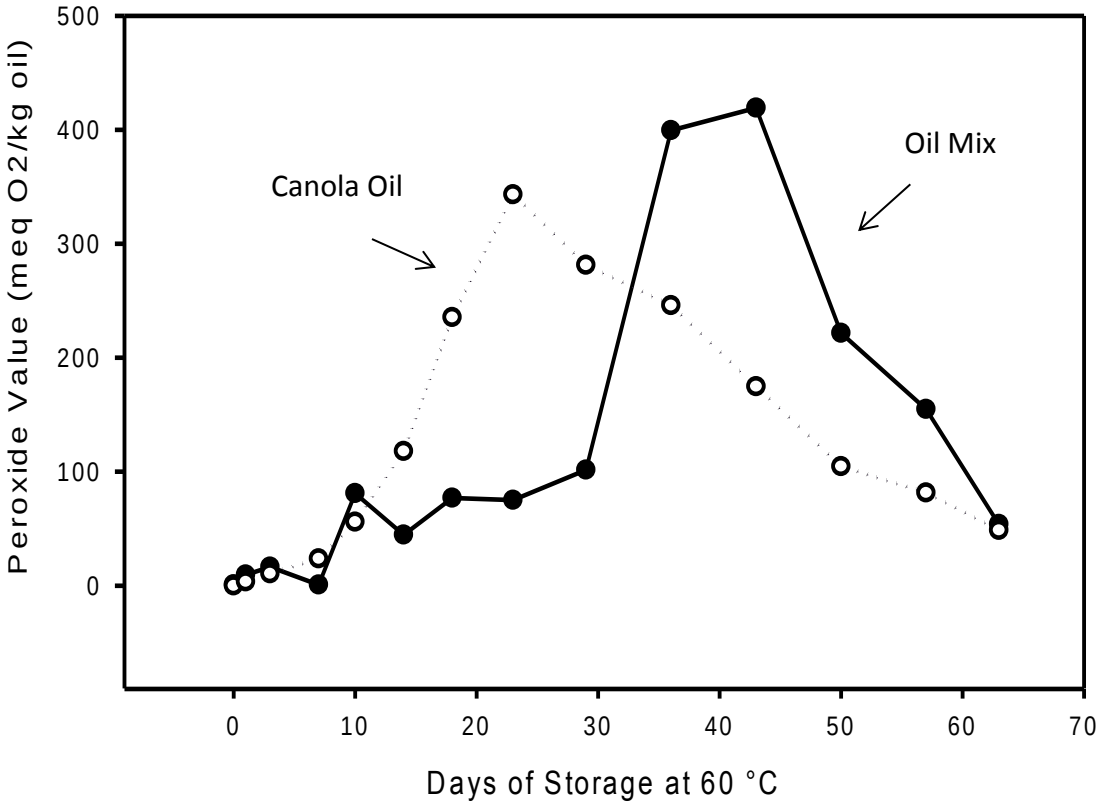


Figure 3.1 Example plot of observed oxidation products over storage time (Sample: Sunflower Oil High Oleic 1)



Analysis	Oil Mix	Canola
Area Under Curve	9772	9524
Maximum Value	419	**343**
Time of Max Value	**43**	23
Time of 20% Max Value	**23**	10
Integrated Area prior to 20% Max Value	1062	**203**
Maximum Value/Time of Max	**9.8**	14.9
Slope of Tangent Line During Growth Phase	19.9	22.8

Figure 3.2 Demonstration of different methods of mathematical interpretations of curves. Numbers in bold depict an indication of superior oxidative stability for the associated sample.

Table 3.1 Oil sample set

Sample	Calculated Iodine Value[a]
MCTs 1	0
MCTs 2	0
Coconut Oil	0.1
Cocoa Butter Substitute	0.15
Hydrogenated Oil Blend	1.33
Palm Kernel Stearin Oil	6.25
Hydrogenated Palm Blend	6.71
Coconut Oil	8.45
Palm Kernel Oil	16.2
Palm Kernel Olein Oil	22.5
Palm Stearin Oil	30.7
Cocoa Butter Substitute	31.5
Cocoa Butter Equivalent 1	31.6
Palm/Coconut Mix	31.6
Cocoa Butter Equivalent 2	31.9
Cocoa Butter Equivalent 3	32.1
Shea Oil/Palm oil blend	40.2
Palm Oil 1	42.1
Palm Oil 2	42.1
Palm Oil 3	47.8
Palm/Coconut High Oleic	47.9
Palm Olein Oil 1	48.1
Cocoa Butter Replacer	49
Palm Oil 4	50
Palm Olein Oil 2	52
Palm Olein Oil 3	53.7
Palm Olein Oil 4	54.5
Palm/Soybean/Canola	57.1
Palm Olein Oil 5	57.2
Palm Olein Oil 6	58.6
Oil Mix A	62.5
Oil Mix B	64.6
Olive Oil 1	74.9
Olive Oil 2	76.6
Sunflower Oil High Oleic 1	81.7
Sunflower Oil High Oleic 2	86
Oil Mix C	94.9
Canola Oil 1	95.4
Corn/Canola Mix	96.3
Corn/Canola Mix D	96.4
Canola/Sunflower/Corn	97.6
Canola Oil 2	103.2
Canola Oil 3	104
Corn Oil 1	107
Corn/Canola Mix E	109
Corn Oil 2	110
Corn Oil 3	111
Corn Oil 4	118
Sunflower Oil	120
Soybean Oil	120

[a]: Determined according to fatty acid compositions determined previously by gas chromatographic analysis, calculated according to AOCS official method Cd 1c-85 (AOCS, 1998)

Table 3.2 Area under the curve values of samples by assay

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	58	120	135	39
MCTs 2	13	96	77	23
Coconut Oil	222	69	71	13
Cocoa Butter Substitute	212	69	93	13
Hydrogenated Oil Blend	163	96	143	29
Palm Kernel Stearin Oil	58	77	24	42
Hydrogenated Palm Blend	175	91	73	36
Coconut Oil	103	87	249	117
Palm Kernel Oil	460	46	350	228
Palm Kernel Olein Oil	1328	154	92	70
Palm Stearin Oil	449	208	281	323
Cocoa Butter Substitute	203	50	175	105
Cocoa Butter Equivalent 1	386	140	575	71
Palm/Coconut Mix	1727	269	630	212
Cocoa Butter Equivalent 2	806	201	458	475
Cocoa Butter Equivalent 3	1253	253	521	436
Shea Oil/Palm oil blend	2117	108	418	534
Palm Oil 1	1412	297	251	187
Palm Oil 2	1521	43	314	134
Palm Oil 3	2261	503	759	432
Palm/Coconut High Oleic	3725	660	945	880
Palm Olein Oil 1	2599	749	416	368
Cocoa Butter Replacer	216	208	252	38
Palm Oil 4	3054	602	448	1160
Palm Olein Oil 2	4375	581	819	1856
Palm Olein Oil 3	2687	706	508	1073
Palm Olein Oil 4	7101	702	1266	808
Palm/Soybean/Canola	7606	162	1175	673
Palm Olein Oil 5	5248	968	755	1714
Palm Olein Oil 6	5913	560	1062	1554
Oil Mix A	4938	412	1031	934
Oil Mix B	7371	1000	2829	2660
Olive Oil 1	6104	493	1757	635
Olive Oil 2	3967	510	2763	2784
Sunflower Oil High Oleic 1	7578	607	1015	697
Sunflower Oil High Oleic 2	8587	854	1523	2658
Oil Mix C	9772	1904	3521	3145
Canola Oil 1	9524	1278	2926	6195
Corn/Canola Mix	6066	1330	4292	2483
Corn/Canola Mix D	10129	1508	4564	3536
Canola/Sunflower/Corn	10026	1927	4207	2574
Canola Oil 2	4005	907	5411	4256
Canola Oil 3	5757	872	4724	5827
Corn Oil 1	11890	1900	2275	1314
Corn/Canola Mix E	9322	2095	4853	3520
Corn Oil 2	11939	1577	2811	2414
Corn Oil 3	9618	1313	5265	5701
Corn Oil 4	10793	1985	2721	4459
Sunflower Oil	8587	1159	4583	5718
Soybean Oil	5372	1505	5240	2040

Table 3.3 Maximum values of samples by assay

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	2	4	4	2
MCTs 2	1	2	3	1
Coconut Oil	10	2	3	1
Cocoa Butter Substitute	10	1	4	0
Hydrogenated Oil Blend	7	3	6	1
Palm Kernel Stearin Oil	3	2	1	2
Hydrogenated Palm Blend	5	3	14	6
Coconut Oil	4	2	12	4
Palm Kernel Oil	39	1	17	19
Palm Kernel Olein Oil	138	6	3	4
Palm Stearin Oil	17	6	16	32
Cocoa Butter Substitute	10	1	10	8
Cocoa Butter Equivalent 1	13	3	60	6
Palm/Coconut Mix	45	8	24	12
Cocoa Butter Equivalent 2	28	4	14	44
Cocoa Butter Equivalent 3	106	5	20	53
Shea Oil/Palm oil blend	79	3	19	34
Palm Oil 1	68	10	6	74
Palm Oil 2	98	2	18	8
Palm Oil 3	70	21	25	19
Palm/Coconut High Oleic	93	15	26	30
Palm Olein Oil 1	109	29	13	87
Cocoa Butter Replacer	10	5	13	6
Palm Oil 4	114	22	11	81
Palm Olein Oil 2	185	16	27	96
Palm Olein Oil 3	132	21	16	95
Palm Olein Oil 4	316	19	82	81
Palm/Soybean/Canola	311	7	75	98
Palm Olein Oil 5	190	24	20	172
Palm Olein Oil 6	357	21	36	184
Oil Mix A	207	12	51	74
Oil Mix B	367	44	115	229
Olive Oil 1	259	14	86	37
Olive Oil 2	173	17	113	154
Sunflower Oil High Oleic 1	360	17	47	61
Sunflower Oil High Oleic 2	351	25	65	165
Oil Mix C	419	70	144	186
Canola Oil 1	343	31	96	220
Corn/Canola Mix	338	44	154	260
Corn/Canola Mix D	422	54	128	159
Canola/Sunflower/Corn	420	63	200	231
Canola Oil 2	97	40	157	207
Canola Oil 3	307	22	155	198
Corn Oil 1	466	60	96	238
Corn/Canola Mix E	569	65	178	382
Corn Oil 2	498	49	112	328
Corn Oil 3	348	34	172	232
Corn Oil 4	386	70	74	397
Sunflower Oil	406	36	153	159
Soybean Oil	350	59	138	69

Table 3.4 Time of maximum values of samples by assay (days of storage at 60 °C)

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	63	36	29	18
MCTs 2	63	7	29	14
Coconut Oil	50	36	36	18
Cocoa Butter Substitute	57	36	18	7
Hydrogenated Oil Blend	63	36	29	43
Palm Kernel Stearin Oil	3	36	29	57
Hydrogenated Palm Blend	29	50	57	57
Coconut Oil	36	43	57	50
Palm Kernel Oil	63	63	57	57
Palm Kernel Olein Oil	63	57	57	57
Palm Stearin Oil	29	63	57	57
Cocoa Butter Substitute	63	18	57	57
Cocoa Butter Equivalent 1	50	57	57	57
Palm/Coconut Mix	29	63	43	57
Cocoa Butter Equivalent 2	57	63	43	57
Cocoa Butter Equivalent 3	63	14	43	57
Shea Oil/Palm oil blend	29	50	57	57
Palm Oil 1	43	50	57	57
Palm Oil 2	57	10	57	57
Palm Oil 3	43	63	29	57
Palm/Coconut High Oleic	43	63	57	57
Palm Olein Oil 1	57	57	57	57
Cocoa Butter Replacer	57	18	57	57
Palm Oil 4	50	57	50	57
Palm Olein Oil 2	50	57	36	36
Palm Olein Oil 3	63	43	57	57
Palm Olein Oil 4	36	57	57	50
Palm/Soybean/Canola	57	50	57	57
Palm Olein Oil 5	43	43	57	57
Palm Olein Oil 6	63	63	36	57
Oil Mix A	50	57	57	57
Oil Mix B	36	43	43	50
Olive Oil 1	36	43	43	43
Olive Oil 2	50	57	57	57
Sunflower Oil High Oleic 1	36	43	57	50
Sunflower Oil High Oleic 2	36	43	43	57
Oil Mix C	43	36	57	57
Canola Oil 1	23	23	29	43
Corn/Canola Mix	36	36	43	57
Corn/Canola Mix D	29	43	43	50
Canola/Sunflower/Corn	43	43	57	57
Canola Oil 2	50	23	29	36
Canola Oil 3	23	18	57	57
Corn Oil 1	43	50	50	57
Corn/Canola Mix E	43	43	50	57
Corn Oil 2	43	43	57	57
Corn Oil 3	36	43	57	43
Corn Oil 4	43	50	57	50
Sunflower Oil	18	18	57	57
Soybean Oil	23	50	36	29

Table 3.5 Time of 20% maximum values of samples by assay (days of storage at 60 °C)

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	0	0	3	0
MCTs 2	18	0	7	0
Coconut Oil	3	0	14	1
Cocoa Butter Substitute	1	0	0	0
Hydrogenated Oil Blend	7	0	1	3
Palm Kernel Stearin Oil	0	0	7	1
Hydrogenated Palm Blend	0	0	36	1
Coconut Oil	7	0	14	3
Palm Kernel Oil	14	0	1	1
Palm Kernel Olein Oil	43	7	7	10
Palm Stearin Oil	10	0	10	10
Cocoa Butter Substitute	10	0	10	1
Cocoa Butter Equivalent 1	1	0	36	10
Palm/Coconut Mix	10	0	10	14
Cocoa Butter Equivalent 2	14	0	0	43
Cocoa Butter Equivalent 3	50	0	0	43
Shea Oil/Palm oil blend	14	0	3	1
Palm Oil 1	18	10	0	43
Palm Oil 2	14	0	10	1
Palm Oil 3	14	10	0	3
Palm/Coconut High Oleic	10	0	0	0
Palm Olein Oil 1	14	14	1	50
Cocoa Butter Replacer	10	0	3	43
Palm Oil 4	18	14	0	29
Palm Olein Oil 2	10	3	3	23
Palm Olein Oil 3	18	10	1	29
Palm Olein Oil 4	23	10	29	29
Palm/Soybean/Canola	14	7	3	29
Palm Olein Oil 5	3	7	1	29
Palm Olein Oil 6	23	3	0	43
Oil Mix A	18	0	0	23
Oil Mix B	23	14	14	29
Olive Oil 1	23	3	10	23
Olive Oil 2	23	3	18	23
Sunflower Oil High Oleic 1	23	10	18	29
Sunflower Oil High Oleic 2	18	10	14	23
Oil Mix C	23	14	7	14
Canola Oil 1	10	3	14	10
Corn/Canola Mix	14	18	0	43
Corn/Canola Mix D	14	10	0	23
Canola/Sunflower/Corn	18	14	0	23
Canola Oil 2	3	3	0	10
Canola Oil 3	10	3	3	7
Corn Oil 1	18	10	1	43
Corn/Canola Mix E	29	14	0	29
Corn Oil 2	18	10	14	29
Corn Oil 3	14	10	14	14
Corn Oil 4	18	18	0	23
Sunflower Oil	7	3	18	7
Soybean Oil	10	10	0	10

Table 3.6 Integrated areas prior to 20% maximum value of samples by assay

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	0	0	1	0
MCTs 2	1	0	3	0
Coconut Oil	4	0	4	0
Cocoa Butter Substitute	1	0	0	0
Hydrogenated Oil Blend	3	0	0	0
Palm Kernel Stearin Oil	0	0	1	0
Hydrogenated Palm Blend	0	0	14	0
Coconut Oil	1	0	2	0
Palm Kernel Oil	25	0	0	2
Palm Kernel Olein Oil	206	4	3	1
Palm Stearin Oil	13	0	18	40
Cocoa Butter Substitute	11	0	11	1
Cocoa Butter Equivalent 1	1	0	21	5
Palm/Coconut Mix	45	0	20	23
Cocoa Butter Equivalent 2	52	0	0	150
Cocoa Butter Equivalent 3	346	0	0	49
Shea Oil/Palm oil blend	63	0	7	3
Palm Oil	53	9	0	110
Palm Oil	33	0	18	1
Palm Oil 3	70	28	0	11
Palm/Coconut High Oleic	84	0	0	0
Palm Olein Oil 1	136	30	1	368
Cocoa Butter Replacer	15	0	0	30
Palm Oil 4	218	26	0	61
Palm Olein Oil 2	103	5	3	85
Palm Olein Oil 3	102	14	0	152
Palm Olein Oil 4	350	21	184	65
Palm/Soybean/Canola	240	6	25	252
Palm Olein Oil 5	41	14	1	260
Palm Olein Oil 6	126	6	0	264
Oil Mix A	153	0	0	285
Oil Mix B	544	52	124	426
Olive Oil 1	385	5	59	70
Olive Oil 2	482	7	92	126
Sunflower Oil High Oleic 1	468	21	27	56
Sunflower Oil High Oleic 2	460	29	14	118
Oil Mix C	1062	96	147	118
Canola Oil 1	203	9	87	109
Corn/Canola Mix	76	65	0	618
Corn/Canola Mix D	219	36	0	236
Canola/Sunflower/Corn	527	78	0	362
Canola Oil 2	25	10	0	147
Canola Oil 3	255	9	31	45
Corn Oil 1	552	23	10	597
Corn/Canola Mix E	1424	94	0	781
Corn Oil 2	301	18	235	320
Corn Oil 3	128	26	118	84
Corn Oil 4	359	89	0	114
Sunflower Oil	314	13	167	66
Soybean Oil	76	50	0	91

Table 3.7 (Maximum value)/(time of maximum value) of samples by assay

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	0.03	0.10	0.14	0.00
MCTs 2	0.02	0.25	0.10	0.00
Coconut Oil	0.21	0.05	0.08	0.01
Cocoa Butter Substitute	0.18	0.03	0.19	0.00
Hydrogenated Oil Blend	0.11	0.08	0.19	0.34
Palm Kernel Stearin Oil	0.99	0.06	0.02	0.14
Hydrogenated Palm Blend	0.18	0.05	0.25	0.05
Coconut Oil	0.10	0.04	0.21	0.40
Palm Kernel Oil	0.61	0.02	0.29	1.62
Palm Kernel Olein Oil	2.19	0.10	0.06	1.02
Palm Stearin Oil	0.58	0.09	0.28	40.36
Cocoa Butter Substitute	0.15	0.07	0.17	0.66
Cocoa Butter Equivalent 1	0.25	0.04	1.06	5.46
Palm/Coconut Mix	1.57	0.12	0.55	23.26
Cocoa Butter Equivalent 2	0.48	0.04	0.32	149.57
Cocoa Butter Equivalent 3	1.68	0.16	0.47	49.40
Shea Oil/Palm oil blend	2.73	0.05	0.33	2.81
Palm Oil 1	1.59	0.18	0.10	109.51
Palm Oil 2	1.72	0.12	0.32	0.54
Palm Oil 3	1.62	0.29	0.86	10.74
Palm/Coconut High Oleic	2.16	0.21	0.46	0.00
Palm Olein Oil 1	1.91	0.47	0.23	367.77
Cocoa Butter Replacer	0.17	0.25	0.23	29.88
Palm Oil 4	2.29	0.34	0.21	60.90
Palm Olein Oil 2	3.71	0.25	0.75	84.78
Palm Olein Oil 3	2.10	0.44	0.28	151.97
Palm Olein Oil 4	8.77	0.30	1.45	64.63
Palm/Soybean/Canola	5.46	0.11	1.31	251.74
Palm Olein Oil 5	4.42	0.48	0.34	260.45
Palm Olein Oil 6	5.67	0.30	1.00	263.56
Oil Mix A	4.14	0.21	0.90	284.52
Oil Mix B	10.19	1.00	2.67	425.65
Olive Oil 1	7.21	0.28	2.00	69.63
Olive Oil 2	3.46	0.24	1.97	126.32
Sunflower Oil High Oleic 1	10.00	0.34	0.82	56.10
Sunflower Oil High Oleic 2	9.74	0.50	1.52	118.14
Oil Mix C	9.75	1.91	2.53	118.46
Canola Oil 1	14.92	1.17	3.31	109.01
Corn/Canola Mix	9.38	1.05	3.59	617.67
Corn/Canola Mix D	14.56	1.15	2.99	236.08
Canola/Sunflower/Corn	9.77	1.42	3.51	362.48
Canola Oil 2	1.95	1.48	5.40	146.97
Canola Oil 3	13.33	1.07	2.72	45.19
Corn Oil 1	10.83	1.05	1.91	597.34
Corn/Canola Mix E	13.24	1.48	3.55	780.95
Corn Oil 2	11.58	0.92	1.96	319.83
Corn Oil 3	9.68	0.58	3.01	83.97
Corn Oil 4	8.97	1.23	1.29	113.52
Sunflower Oil	22.57	1.67	2.68	65.79
Soybean Oil	15.20	0.86	3.84	91.26

Table 3.8 Slopes of tangent lines during growth phase of samples by assay

Sample	PV	CDT	TBARS	*p*-AnV
MCTs 1	0.0	0.3	0.1	0.3
MCTs 2	0.1	0.1	0.2	0.2
Coconut Oil	0.7	0.1	0.1	0.1
Cocoa Butter Substitute	0.4	0.0	0.3	0.0
Hydrogenated Oil Blend	0.3	0.2	0.6	0.1
Palm Kernel Stearin Oil	0.9	0.0	0.0	0.0
Hydrogenated Palm Blend	0.1	0.1	0.6	0.0
Coconut Oil	0.1	0.0	0.3	0.1
Palm Kernel Oil	1.6	0.0	0.3	0.1
Palm Kernel Olein Oil	6.1	0.1	0.0	0.1
Palm Stearin Oil	0.8	0.1	0.3	0.3
Cocoa Butter Substitute	0.4	0.0	0.5	0.0
Cocoa Butter Equivalent 1	0.2	0.0	2.8	0.1
Palm/Coconut Mix	1.6	0.7	0.8	0.7
Cocoa Butter Equivalent 2	1.0	0.2	0.5	2.9
Cocoa Butter Equivalent 3	7.5	0.2	1.1	3.7
Shea Oil/Palm oil blend	4.3	0.0	0.2	0.3
Palm Oil 1	2.1	0.2	0.1	5.0
Palm Oil 2	2.2	0.1	0.3	0.1
Palm Oil 3	1.6	0.6	1.5	0.9
Palm/Coconut High Oleic	2.2	0.2	0.3	1.1
Palm Olein Oil 1	1.7	0.4	0.1	10.0
Cocoa Butter Replacer	0.3	0.3	0.2	0.4
Palm Oil 4	2.7	0.3	0.2	2.1
Palm Olein Oil 2	3.6	0.2	0.4	7.0
Palm Olein Oil 3	2.9	0.4	0.2	3.0
Palm Olein Oil 4	21.0	0.3	2.3	3.6
Palm/Soybean/Canola	6.8	0.1	1.4	2.4
Palm Olein Oil 5	3.7	0.5	0.2	5.2
Palm Olein Oil 6	39.1	0.2	2.0	12.8
Oil Mix A	5.8	0.2	1.4	1.8
Oil Mix B	24.1	1.1	3.6	10.1
Olive Oil 1	17.2	0.2	1.7	1.6
Olive Oil 2	5.3	0.2	2.7	3.8
Sunflower Oil High Oleic 1	23.9	0.4	0.9	2.6
Sunflower Oil High Oleic 2	17.9	0.5	2.0	4.8
Oil Mix C	19.9	2.5	3.4	4.0
Canola Oil 1	22.8	1.2	5.7	4.6
Corn/Canola Mix	38.3	1.6	8.1	16.8
Corn/Canola Mix D	21.4	1.6	2.2	5.3
Canola/Sunflower/Corn	22.5	1.9	3.6	5.5
Canola Oil 2	10.7	2.6	4.5	12.4
Canola Oil 3	19.5	1.1	1.7	3.1
Corn Oil 1	13.2	1.2	1.0	14.8
Corn/Canola Mix E	34.1	3.3	3.9	10.8
Corn Oil 2	14.6	0.9	1.8	10.7
Corn Oil 3	15.7	0.5	3.7	7.7
Corn Oil 4	13.7	1.3	1.2	13.2
Sunflower Oil	28.6	1.8	2.3	3.6
Soybean Oil	19.9	2.1	4.5	4.0

Table 3.9 Average deviation across assays (ADAA) according to numerical interpretation technique

Sample	ADAA
Area Under Curve	3.98
Maximum Value	4.26
Time of Max Value	10.4
Time of 20% Max Value	9.48
Integrated Area prior to 20% Max Value	8.22
Maximum Value/Time of Max	5.72
Slope of Tangent Line During Growth Phase	5.99

Table 3.10 St. Sum AUC of fat and oil samples

Sample	St. Sum AUC
MCTs 1	320
MCTs 2	221
Coconut Oil	220
Cocoa Butter Substitute	232
Hydrogenated Oil Blend	306
Palm Kernel Stearin Oil	182
Hydrogenated Palm Blend	261
Coconut Oil	409
Palm Kernel Oil	579
Palm Kernel Olein Oil	685
Palm Stearin Oil	855
Cocoa Butter Substitute	319
Cocoa Butter Equivalent 1	742
Palm/Coconut Mix	1418
Cocoa Butter Equivalent 2	1157
Cocoa Butter Equivalent 3	1364
Shea Oil/Palm oil blend	1354
Palm Oil 1	1119
Palm Oil 2	748
Palm Oil 3	2162
Palm/Coconut High Oleic	3218
Palm Olein Oil 1	2366
Cocoa Butter Replacer	573
Palm Oil 4	2837
Palm Olein Oil 2	3874
Palm Olein Oil 3	2886
Palm Olein Oil 4	4285
Palm/Soybean/Canola	3404
Palm Olein Oil 5	4560
Palm Olein Oil 6	4167
Oil Mix A	3225
Oil Mix B	7168
Olive Oil 1	3901
Olive Oil 2	5590
Sunflower Oil High Oleic 1	4012
Sunflower Oil High Oleic 2	6391
Oil Mix C	9992
Canola Oil 1	10744
Corn/Canola Mix	8186
Corn/Canola Mix D	10415
Canola/Sunflower/Corn	10128
Canola Oil 2	9003
Canola Oil 3	10066
Corn Oil 1	8391
Corn/Canola Mix E	11315
Corn Oil 2	9032
Corn Oil 3	11988
Corn Oil 4	10798
Sunflower Oil	11057
Soybean Oil	8587

CHAPTER 4

MODELING THE RELATIVE EFFECTS OF MUFA, DIUFA, AND TRIUFA ON THE ACCUMULATION OF LIPID AUTOXIDATION PRODUCTS

INTRODUCTION

The oxidative deterioration of lipids in food products can affect food safety, nutrition, texture, color, as well as result in the release of aromatic volatile compounds responsible for undesirable or "rancid" flavors associated with poor food quality (Gray, 1978; Ajuyah et al., 1993; Morales et al., 1997). Many contributing factors to lipid oxidation have been cited including: storage temperature, processing methods, and the concentrations of oxygen, free fatty acids, pro-oxidants, and antioxidants. Paramount among these, however, is the fatty acid composition of the sample – or more specifically, the sample's unsaturation. This is logical given that oxidative reactions initiate upon carbon-carbon double bonds (Parker, 2003). In most cases, higher proportions of unsaturates inherent to lipids will lead both to more rapid autoxidation as well as to a greater accumulation of lipid oxidation products (Martin-Polvillo et al., 2004). Given this observed correlation, the simple measure of iodine value (an analytical measure for the ascertainment of the quantity of double bonds present) of lipids can often serve as a fair, yet still not entirely consistent, indicator of the lipids oxidative stability (Tan et al., 2002). Other studies have investigated the importance of double bond distribution within fatty acids and found fatty acids with higher degrees of unsaturation oxidize more quickly (Min and Bradley, 2002).

However, the observed effects of these factors in scientific study have been inconsistent, and studies have encountered significant deviations from the simple expectation that more unsaturated samples will generally show lesser oxidative stability (Min and Boff, 2002; Tan et al., 2002). One possible explanation may lie in the inconsistent and/or incomprehensive assessments and definitions of oxidative stability. To address this concern, this study employs a large-scale and highly comprehensive study design (i.e. 50 samples, four common methods to detect oxidation products, two months of accelerated storage, and greater than 100 data points per sample), and consolidates the data into a single quantitative summation of oxidative stability for each sample (the Standardized Summation of Area under the Curve; see chapter 3). The quantified term of oxidative stability is compared to the unsaturation of the samples, both by simple Calculated Iodine Value (CIV), and also as delineated by the content of fatty acids containing either one, two, or three double bonds. The objective is to produce a rigorous assessment of what proportion of oxidative stability behavior can be meaningfully attributed to the unsaturation of a sample, and also to determine the importance of the combined presence of multiple double bonds on individual fatty acids upon oxidative stability

MATERIALS AND METHODS

Samples: Selection and Handling

50 commercially available fat and oil samples were provided by the Nestlé Research Center/NESTEC Ltd. (Lausanne, Switzerland), see **Table 4**. This table also includes the samples' Calculated Iodine Values (CIV; a comprehensive measure of total unsaturation within a fat or oil). All samples were verified by HPLC analysis to be free of added synthetic antioxidants, according to AOAC official method 983.15.

Oxidation data

Acquisition of oxidation data used for this chapter is described in chapter 3 of this dissertation.

Fatty Acid Composition

Fatty acids were quantified by gas chromatography, in accordance with the GC-FID-FAME procedure outlined in (Badings and De Jong, 1988). Concentrations of MUFA (fatty acids with one double bond), DiUFA (fatty acids with two double bonds), and TriUFA (fatty acids with three double bonds) were calculated directly from this data.

Calculated Iodine Value

To determine summations of total unsaturation within the samples, Calculated Iodine Values were evaluated according to AOCS official method Cd 1c-85 (AOCS, 1998). This calculation sums the proportional content of all unsaturated fatty acids, each multiplied by a factor accounting for both the number of double bonds within the compound as well as its molecular weight. The results are reported as g iodine/100 g oil in reference to the traditional iodine value assay of which these results are intended to closely approximate.

Standardized Summation of Area under the Curve

For a comprehensive quantification of oxidative stability within samples, the "Standardization Summation of Area under the Curve" (St. Sum AUC) term was calculated from the curves of the four oxidative product assays. This derived term is proposed to be a meaningful and comprehensive quantitative summary of an oil or fat's oxidative stability. The

derivation of this term (as well as the justification for its use) can be found in chapter 3 of this dissertation. The AUC values were computed using integral calculus computations within Sigmaplot 12.0 (Systat Software, San Jose, CA), and the St. Sum AUC values were calculated according to the following equation (1):

$$\text{St. Sum AUC} = [PV_{AUC} + (6.31)CDT_{AUC} + (2.60)TBARS_{AUC} + (2.87)p\text{-}AnV_{AUC}]/4 \quad (1)$$

Multiple Linear Regression

The linear regression analysis for this study was performed using SAS software according to the REG procedure (Copyright, SAS Institute Inc).

RESULTS AND DISCUSSION

Quantification of Fatty Acids

Table 4.2 shows the concentrations of MUFA, DiUFA, and TriUFA. MUFA were generally most abundant in oils of medium unsaturation (i.e. CIV of approximately 50-90), and were of the highest concentrations in olive oil samples and high oleic sunflower oil samples. These are in accordance with expectations, given the high concentrations of oleic acid (18:1n-9) expected in these oil-types (Perez-Jimenez et al., 1995; Stark and Madar, 2002).

DiUFA were most abundant in highly unsaturated oils, and were generally highest in samples of corn oil, sunflower oil, and soybean oil. This is expected, considering these oils' known tendency to contain high quantities of linoleic acid (18:2n-6) (Dupont et al., 1990; Kris-Etherton et al., 2000; Miller et al., 1987). Canola oils exhibited relatively (in consideration of their total sample unsaturation) low concentrations of DiUFA.

TriUFA were most abundant in more unsaturated oils, but were of concentrations that did not consistently correlate to increasing unsaturation. For example, Corn Oil 1, Corn Oil 2, and Soybean Oil were among the two most highly unsaturated samples yet contained very small quantities of TriUFA. Canola oils (and blends including canola oil) were the most abundant sources of TriUFA. Canola oils are known for their relatively high quantities of of α-linolenic acid (18:3n-3), so this result agrees with expectations (Freese et al., 1994; Mozaffarian, 2004).

Standardized Summation of Area under the Curve

Table 4.3 shows the St. Sum AUC values of the 50 samples. The data shows a clear, but inconsistent, trend towards higher St. Sum AUC values in accordance with greater unsaturation. This is in accordance with expectations. Notable exceptions to this trend include the Cocoa Butter Replacer sample and the Palm Oil 2 both of which exhibit much greater stability than would be expected according to their CIV. Oil Mix B exhibits lesser oxidative stability than would be expected.

Correlation between St. Sum AUC and CIV

Figure 4.1 depicts the correlation between St. Sum AUC values and CIV. The equation of this model is as follows (2):

$$\text{St. Sum AUC} = -1382 + (99.3)\text{CIV} \qquad (2)$$

The correlation ($R^2 = 87.3\%$) is quite strong, and suggests a good deal of predictive power of stability according to only the simple measure of CIV. The association is in accordance with expectations, but the strength of the association is greater than that observed in many previous

studies. This greater strength of association may be attributable to the comprehensive nature of the St. Sum AUC term, which has been designed to minimize the occurrence of outlier behavior that may result from the specificities of particular assays, study designs, or interpretation techniques. It should be noted, however, that the correlation is perhaps somewhat inflated by simple magnitude-to-magnitude comparisons. The predictive power of this model clearly diminishes when making comparisons within small ranges of CIV.

Correlation between St. Sum AUC and MUFA, DiUFA, and TriUFA

Figure 4.2 depicts the correlation between St. Sum AUC values and the concentrations MUFA, DiUFA, and TriUFA. The equation of the model is as follows (3):

$$\text{St. Sum AUC} = -618 + 53.2[\text{MUFA}] + 167[\text{DiUFA}] + 635[\text{TriUFA}] \quad (3)$$

. The correlation (R^2_{adj} = 91.5%) is very strong, and all three independent variables are statistically significant ($\alpha = 0.05$). The correlation of this model is a notable improvement upon the model based upon only the simple measure of CIV. This suggests that oxidative stability may be better predicted with the consideration of the distribution of unsaturation among fatty acids in a sample.

According to this model, the presence of MUFA, DiUFA, and TriUFA each impairs a sample's stability. Interestingly, the ratio of their relative effect upon stability (as quantified by their coefficients in the model) is approximately 1:3:12 (specifically, 53.2:167:635) – substantially different than the 1:2:3 ratio of their relative unsaturation. This suggests a possible synergistic oxidative effect attributable to the combined presence of multiple double bonds on individual fatty acids. This effect may be due to a breakdown of stable energy distribution

within a fatty acid upon the initiation of oxidative reactions. This result is not unprecedented (Martin-Polvillo et al., 2004), but has not been consistently or frequently quantified with rigor (Jones, 1994; Leyton et al., 1987; McCormick et al., 2007).

Certain controlled studies have stimulated oxidation and have found lipid oxidation rates associate linearly not with the double bonds, but with the the total number of bis-allylic sites (the methylene CH directly adjacent to two double bonds) (Cosgrove et al., 1987; McCormick et al., 2007). The single allylic site has been found to be much less reactive, which could explain the observation here of the relatively low contribution to oxidation of MUFA. The ratio of bisallylic sites in DiUFA and TriUFA (1:2) is closer to our observed ratio of oxidative impact than the ratio of double bonds (2:3), but still underestimates the differences in oxidation observed in this study (1:4). It should be considered, though, that the 1:2 expectation of DiUFA and TriUFA is in regards to initial reaction rate, and not accumulation of oxidation products. Given the possible capabilities of intial oxidation reaction products to serve as pro-oxidants, it is conceivable the greater presences of TriUFAs are not only themselves oxidizing rapidly, but also in so doing simultaneously producing a less favorable environment in regards to oxidative stability (Chaiyasit et al., 2007; Kim et al., 2007, Morita and Fujimaki, 1973; Morita et al., 1976; Morita and Tokita 2006). The effect of such an action could well be one of intensified oxidative instability, as observed in this study.

CONCLUSIONS

It was determined that the concentrations of monounsaturated fatty acids (MUFA), diunsaturated fatty acids (DiUFA), and triunsaturated fatty acids (TriUFA) were all statistically significant predictors of oxidation, and together demonstrated a strong correlation (R^2_{adj} =

91.5%) with the measure of exhibited accumulation of oxidation products. This model outperformed a model created using CIV as the sole independent variable (R^2_{adj} = 87.3%). The model of concentrations MUFA, DiUFA, and TriUFA indicated the relative effect upon oxidative stability of MUFA:DiUFA:TriUFA to be approximately 1:3:12 – substantially greater than that of their relative degrees of unsaturation. The results suggest that the combined presence of multiple double bonds on individual fatty acids is associated with impaired oxidative stability, even more so than a comparison of bis-allylic sites would predict. It is speculated that TriUFA may impart to long-term oxidative stability detrimental effects beyond that of simply presenting a high number of possible reaction sites for oxidation. In addition to providing a model for oxidative stability with good predictive strength, the results of this study indicate possible basis of preference for the use of oils and fats that attain their level of unsaturation (and the nutritional and functional properties therein) from double bonds more evenly distributed across their fatty acids (e.g. more MUFA, and fewer TriUFA).

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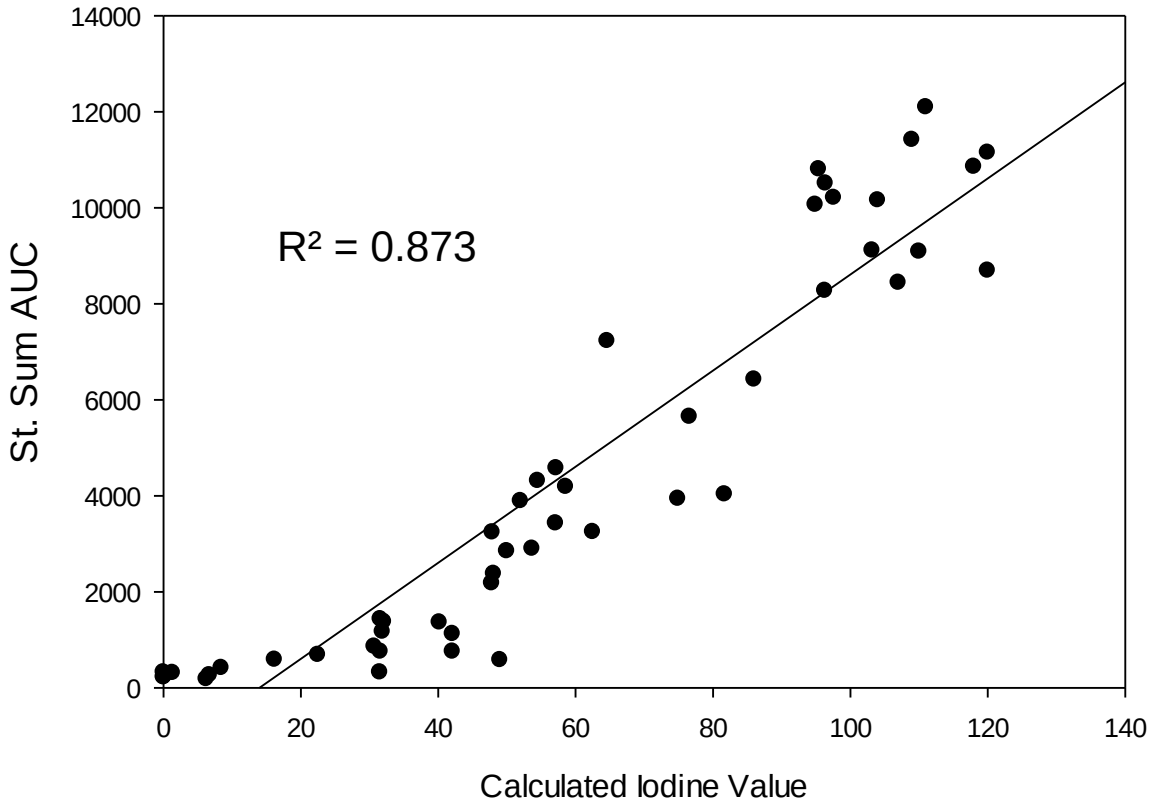

Figure 4.1 Correlation between St. Sum AUC (term for oxidative stability) and CIV (indicative of sample unsaturation)

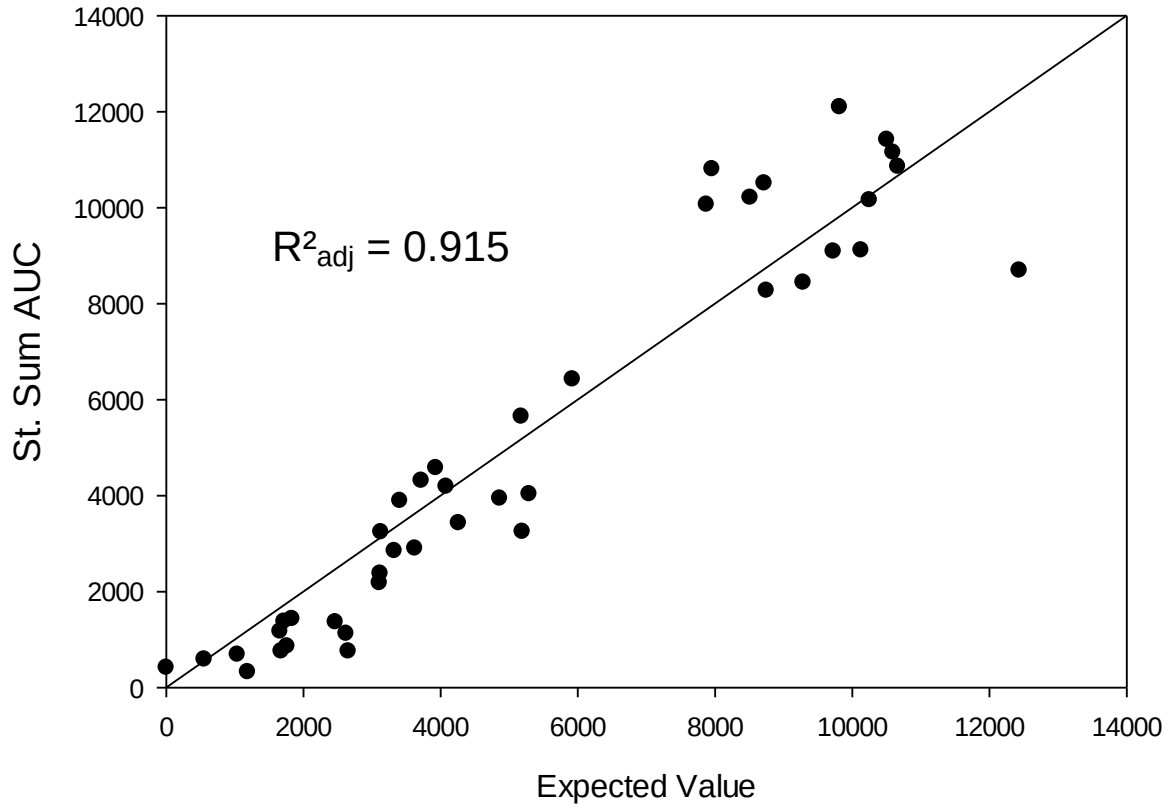

Figure 4.2 Correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA

Table 4.1 Oil sample set

Sample	Calculated Iodine Value[a]
MCTs 1	0
MCTs 2	0
Coconut Oil	0.1
Cocoa Butter Substitute	0.15
Hydrogenated Oil Blend	1.33
Palm Kernel Stearin Oil	6.25
Hydrogenated Palm Blend	6.71
Coconut Oil	8.45
Palm Kernel Oil	16.2
Palm Kernel Olein Oil	22.5
Palm Stearin Oil	30.7
Cocoa Butter Substitute	31.5
Cocoa Butter Equivalent 1	31.6
Palm/Coconut Mix	31.6
Cocoa Butter Equivalent 2	31.9
Cocoa Butter Equivalent 3	32.1
Shea Oil/Palm blend	40.2
Palm Oil 1	42.1
Palm Oil 2	42.1
Palm Oil 3	47.8
Palm/Coconut High Oleic	47.9
Palm Olein Oil 1	48.1
Cocoa Butter Replacer	49
Palm Oil 4	50
Palm Olein Oil 2	52
Palm Olein Oil 3	53.7
Palm Olein Oil 4	54.5
Palm/Soybean/Canola	57.1
Palm Olein Oil 5	57.2
Palm Olein Oil 6	58.6
Oil Mix A	62.5
Oil Mix**B**	64.6
Olive Oil 1	74.9
Olive Oil 2	76.6
Sunflower Oil High Oleic 1	81.7
Sunflower Oil High Oleic 2	86
Oil Mix C	94.9
Canola Oil 1	95.4
Corn/Canola Mix	96.3
Corn/Canola Mix D	96.4
Canola/Sunflower/Corn	97.6
Canola Oil 2	103
Canola Oil 3	104
Corn Oil 1	107
Corn/Canola Mix E	109
Corn Oil 2	110
Corn Oil 3	111
Corn Oil 4	118
Sunflower Oil	120
Soybean Oil	120

[a]: Determined according to fatty acid compositions determined previously by gas chromatographic analysis, calculated according to AOCS official method Cd 1c-85 (AOCS, 1998)

Table 4.2 Concentrations of MUFA, DiUFA, and TriUFA within fat and oil samples

Sample	Calculated Iodine Value	MUFA (% FA)	DiUFA (% FA)	TriUFA (% FA)
MCTs 1	0	0.0	0.0	0.0
MCTs 2	0	0.0	0.0	0.0
Coconut Oil	0.1	0.1	0.0	0.0
Cocoa Butter Substitute	0.15	0.1	0.0	0.0
Hydrogenated Oil Blend	1.33	0.8	0.2	0.0
Palm Kernel Stearin Oil	6.25	5.3	0.9	0.1
Hydrogenated Palm Blend	6.71	5.9	0.9	0.0
Coconut Oil	8.45	6.5	1.6	0.0
Palm Kernel Oil	16.2	14.3	2.2	0.0
Palm Kernel Olein Oil	22.5	19.4	3.3	0.0
Palm Stearin Oil	30.7	24.1	5.6	0.1
Cocoa Butter Substitute	31.5	28.6	1.1	0.0
Cocoa Butter Equivalent 1	31.6	30.2	3.1	0.1
Palm/Coconut Mix	31.6	24.2	6.0	0.1
Cocoa Butter Equivalent 2	31.9	30.5	3.2	0.0
Cocoa Butter Equivalent 3	32.1	30.9	3.1	0.1
Shea Oil/Palm	40.2	32.2	7.0	0.1
Palm Oil 1	42.1	33.6	7.3	0.2
Palm Oil 2	42.1	34.6	6.8	0.2
Palm Oil 3	47.8	36.7	9.2	0.1
Palm/Coconut High Oleic	47.9	35.9	9.6	0.1
Palm Olein Oil 1	48.1	37.2	9.1	0.1
Cocoa Butter Replacer	49	43.7	1.6	0.0
Palm Oil 4	50	37.8	9.8	0.2
Palm Olein Oil 2	52	41.6	9.0	0.2
Palm Olein Oil 3	53.7	40.6	10.6	0.2
Palm Olein Oil 4	54.5	40.4	11.2	0.2
Palm/Soybean/Canola	57.1	36.5	13.5	0.9
Palm Olein Oil 5	57.2	41.4	12.1	0.2
Palm Olein Oil 6	58.6	42.8	12.2	0.3
Oil Mix A	62.5	33.5	16.5	2.0
Oil Mix B	64.6	36.2	16.7	1.8
Olive Oil 1	74.9	73.2	6.0	0.5
Olive Oil 2	76.6	70.2	8.5	0.6
Sunflower Oil High Oleic 1	81.7	77.7	8.5	0.0
Sunflower Oil High Oleic 2	86	71.8	13.8	0.1
Oil Mix C	94.9	66.4	16.5	3.5
Canola Oil 1	95.4	66.6	16.6	3.6
Corn/Canola Mix	96.3	40.4	30.5	3.4
Corn/Canola Mix D	96.4	41.6	29.9	3.4
Canola/Sunflower/Corn	97.6	59.0	20.8	4.1
Canola Oil 2	103	58.5	18.7	7.9
Canola Oil 3	104	57.0	19.6	8.0
Corn Oil 1	107	33.6	44.1	0.7
Corn/Canola Mix E	109	43.8	33.4	5.4
Corn Oil 2	110	31.3	47.1	0.8
Corn Oil 3	111	29.0	48.4	0.8
Corn Oil 4	118	26.9	53.4	1.0
Sunflower Oil	120	22.7	48.5	6.4
Soybean Oil	120	29.4	54.6	0.2

Table 4.3 St. Sum AUC of fat and oil samples

Sample	St. Sum AUC
MCTs 1	320
MCTs 2	221
Coconut Oil	220
Cocoa Butter Substitute	232
Hydrogenated Oil Blend	306
Palm Kernel Stearin Oil	182
Hydrogenated Palm Blend	261
Coconut Oil	409
Palm Kernel Oil	579
Palm Kernel Olein Oil	685
Palm Stearin Oil	855
Cocoa Butter Substitute	319
Cocoa Butter Equivalent 1	742
Palm/Coconut Mix	1418
Cocoa Butter Equivalent 2	1157
Cocoa Butter Equivalent 3	1364
Shea Oil/Palm	1354
Palm Oil 1	1119
Palm Oil 2	748
Palm Oil 3	2162
Palm/Coconut High Oleic	3218
Palm Olein Oil 1	2366
Cocoa Butter Replacer	573
Palm Oil 4	2837
Palm Olein Oil 2	3874
Palm Olein Oil 3	2886
Palm Olein Oil 4	4285
Palm/Soybean/Canola	3404
Palm Olein Oil 5	4560
Palm Olein Oil 6	4167
Oil Mix A	3225
Oil Mix B	7168
Olive Oil 1	3901
Olive Oil 2	5590
Sunflower Oil High Oleic 1	4012
Sunflower Oil High Oleic 2	6391
Oil Mix C	9992
Canola Oil 1	10744
Corn/Canola Mix	8186
Corn/Canola Mix D	10415
Canola/Sunflower/Corn	10128
Canola Oil 2	9003
Canola Oil 3	10066
Corn Oil 1	8391
Corn/Canola Mix E	11315
Corn Oil 2	9032
Corn Oil 3	11988
Corn Oil 4	10798
Sunflower Oil	11057
Soybean Oil	8587

CHAPTER 5

SEQUENTIAL APPROACH FOR THE CONSIDERATION OF REDUNDANT FACTORS IN THE MATHEMATICAL MODELING OF OXIDATIVE STABILITY

INTRODUCTION

Redundancy within factors considered presents a major challenge to effective modeling (Bentler and Chou, 1987). Redundancy can occur either directly (both factors depict the same important consideration), or indirectly (both factors show linearity with another affecting factor). In either case, the redundancy can often impede the direct understanding of the "unique" importance of a factor (Motulsky and Christopoulos, 2004).

The data of the study discussed in this dissertation provides an excellent example of this problem. Here, the highly important factor of sample unsaturation is reiterated redundantly by multiple factors in the composition data of the oils and fats, which impairs the process of understanding these factors' possible unique contributions to oxidation. For example, the concentration of an unsaturated fatty acid such as α-linolenic acid (18:3n-3) can safely be expected to show a negative linear relationship with oxidative stability, but this expectation is due to its relatively large contribution of double bonds to the oil sample (Gromadzka *et al*., 2008). Statistical confirmation of this effect does not tell us anything about the specific effect that may be associated uniquely with the configuration of this particular fatty acid.

Efforts to make models according to redundant data run the danger of not only being ineffective, but also highly misleading. Our data provides demonstrations of this effect as well. For example, it stands to reason that samples which contain more double bonds will also contain

higher concentrations of triacylglycerols (TAG) that are unsaturated. This in turn means that any specific degree of unsaturation within a TAG will likely be of higher concentration as well. The effect can be misleading models such as the one that follows (1):

$$\text{St. Sum AUC} = 810 + 339\ \text{TAG 4 double bonds} \quad (1)$$

This model has a strong correlation (R^2 = 88.8%), but its merit is highly dubious. Common sense tells us that the number of TAG containing four double bonds is serving here simply as an indicator of greater sample unsaturation, and we are not learning anything of importance regarding the effect of TAG containing specifically four double bonds. We can somewhat validate this concern by observing that TAG with four double bonds shows a similarly strong correlation (R^2 = 86.6%) to calculated iodine value. In other words, it would clearly be poor judgment to attribute lipid oxidation specifically to the presence of TAG with four double bonds.

Similar problems emerge when we consider factors that are perhaps not directly related to sample unsaturation, but that still suffer dilemmas of meaning because their importance to oxidative stability is so small relative to other considerations. In effect, their true role in an outcome is quantitatively dwarfed by their (oftentimes perhaps coincidental) correlation with more quantitatively important factors. For example, our St. Sum AUC term of oxidative stability shows a significant (R^2 = 36%) positive correlation with the measure of α-tocopherol. Interpreted without skepticism, one could conclude this as evidence that more than one third of lipid autoxidative behavior is positively attributable to the concentration of α-tocopherol. This is, of course, not the case, and further scrutiny reveals that α-tocopherol actually correlates even better with sample unsaturation (R^2 = 44%) than it does with stability (**Figure 5.1**). A more reasonable conclusion is that in our sample set, α-tocopherol generally occurs in higher

concentrations in samples that are more highly unsaturated, which in turn is associated with lesser oxidative stability. This could be coincidental, or indicative of a pattern regarding vitamin content. Regardless, it is clear that the indirect association with higher unsaturation is overwhelming any direct correlatory effect of the compound upon stability. An interpretation of its more direct effects upon oxidative stability would therefore require a more involved statistical approach.

This chapter discusses a systematic sequential statistical approach that is used throughout this dissertation to address this concern. It also shows the ordering of factors that results from this interpretative technique. The technique involves an ordering of factors in sequential steps, according to their respective abilities to correlate with the variance unexplained by the previous best model. The previous best model used as the starting base for this investigation is according to sample unsaturation, and is as follows (2):

$$\text{St. Sum AUC} = -618 + 53.2[\text{MUFA}] + 167[\text{DiUFA}] + 635\,[\text{TriUFA}] \quad (2)$$

Additional factors considered for improvements upon this model include TAG positional isomers, TAG double bond distribution, monoacylglycerol concentration, free fatty acid concentration, fatty acid composition, sample purity (i.e. not a blend), and endogenous vitamin concentration.

MATERIALS AND METHODS

Oxidation data

Acquisition of oxidation data used for this chapter is described in chapter 3 of this dissertation.

Standardized Summation of Area under the Curve

The "Standardization Summation of Area under the Curve" (St. Sum AUC) term was calculated as described in chapter 3 according to the following equation (3):

$$\text{St. Sum AUC} = [PV_{AUC} + (6.31)CDT_{AUC} + (2.60)TBARS_{AUC} + (2.87)p\text{-}AnV_{AUC}]/4 \quad (3)$$

Regioisomeric Distribution of Fatty Acids in Triacylglycerols

Regioisomeric distribution of fatty acids in TAG was determined by hybrid mass spectrometry according to the methodology described by Nagy *et al*. (2012). For each sample, this data was used to calculate positional isomers, monoacylglycerols, free fatty acids, and TAG double bond distribution.

Quantification of Fatty Acids

Fatty acids were quantified by gas chromatography, in accordance with the GC-FID-FAME procedure outlined in Badings and De Jong (1988).

Sample Purity

Sample purity was ascertained according to the specification sheets of the oil suppliers. Here, purity is meant simply to describe "single source" (e.g. canola oil, soybean oil), as in contrast to oil blends (e.g. confectionary blends, palm/coconut blends). It is not a quantitative term, and is treated as a binary variable.

Endogenous Vitamin Concentrations

Endogenous vitamin concentrations of the samples were evaluated by HPLC-MS, according to the method described in Nagy *et al*. (2007). The vitamins assessed were β-carotene, vitamin K, retinyl acetate, α-tocopherol, α-tocotrienol, β–tocopherol, γ-tocopherol, β-tocotrienol, γ-tocotrienol, σ-tocopherol, and σ–tocotrienol.

Determination of Sequential Consideration of Factors

The method for determining ordering of factors consisted of the following steps:

1) Begin with model of St. Sum of AUC, according to concentration of monounsaturated fatty acids (MUFA), diunsaturated fatty acids (DiUFA), and triunsaturated fatty acids (TriUFA). This is due to well-established knowledge of the high importance of unsaturation to stability
2) Compare remaining factors for ability to explain the unexplained variance (i.e. the residuals) of the previous best model.
3) Incorporate factor found in step 2 into previous best model, and create new "best" model using consideration of correlation score and model simplicity.
4) Repeat steps 2 and 3 until remaining factors cease to prove significant predictors of remaining variance ($\alpha = 0.05$).

In all cases, the linear regression analysis for this study was performed using SAS software (copyright, SAS Institute Inc). The 'Best Subsets' procedure was performed to compare possible model outcomes.

RESULTS AND DISCUSSION

Standardized Summation of Area under the Curve

Table 4.3 (refer to chapter 4) shows the St. Sum AUC values of the 50 samples. The data shows a clear, but inconsistent, trend towards higher St. Sum AUC values in accordance with greater unsaturation. This is in accordance with expectations.

Positional Isomers, Monoacylglycerols, and Free Fatty Acid Concentration

Each of these three factors was considered at each step of the model-improvement process, but failed in all cases to show efficacy for the improvement of the models.

TAG Double Bond Distribution, Fatty Acid Composition, Sample Purity, and Endogenous Vitamin Concentrations

Tables 5.1, 5.2, 5.3, and 5.4 show the data for TAG double bond distribution, relevant fatty acids (i.e. *trans*-fatty acids and unsaturated fatty acids longer than 18 carbons), sample purity, and endogenous vitamin concentrations, respectively. These factors were all determined to be useful factors in the sequential efforts to improve the mathematical model

Determination of Sequential Consideration of Factors

The sequential ordering of factors was determined to be as follows: (1) TAG double bond distribution, (2) fatty acid composition, (3) sample purity, and (4) endogenous vitamin concentration.

To help illustrate the process of this determination, **Figure 5.2** shows the initial best model (according to MUFA, DiUFA, and TriUFA), and **Figure 5.3** shows the remaining unexplained

variance of this model. **Table 5.5** shows the comparative correlatory strengths of the four considered factors to this unexplained variance. The table shows that TAG double bond distributions has the greatest correlation to the unexplained variance of the previous best model (R^2_{adj} = 32.7%). The model which corresponds to this correlation is as follows (4):

$$\begin{matrix} \text{Prior} \\ \text{Residual} \end{matrix} = 676 - 23.7[\text{TAG w/ 1 double bond}] - 402[\text{TAG w/ 7 double bonds}] \quad (4)$$

The correlation of this model is shown in **Figure 5.4**. The value of the prior residual signified a proliferation of oxidative products that was not explained by consideration of unsaturation (specifically, MUFA, DiUFA, and TriUFA concentrations). The coefficients of this model therefore signify that the concentration of TAG with one double bond is associated with an improved oxidative stability within the investigated sample set, and the concentration of TAG with seven double bonds is associated with much greater improvements upon stability. Interpretation here, however, requires careful thought, as TAG with seven double bonds only demonstrate this effect in models which already account for the oxidative instability contributed by the presence of seven double bonds. In other words, the unsaturated fatty acids found in a TAG with seven double bonds are still predicted by the model to correlate with oxidative instability but their shared presence upon a single TAG is associated with a diminishment in this effect. Therefore, we believe we are seeing the correlatory effect not of TAG with seven double bonds, exactly, but rather the correlatory effect of seven double bonds being on a single TAG. The distinction is subtle, but is crucially relevant to the objective of isolating the possible unique effects of a factor from its other, possibly redundant, effects.

The next step of our procedure is to incorporate the new selected variables into our previous best model, which yields the following (5):

$$\text{St. Sum AUC} = 58 + 47.9[\text{MUFA}] + 168[\text{DiUFA}] + 883[\text{TriUFA}] - 19.3[\text{TAG w/ 1 double bond}] - 759[\text{TAG w/ 7 double bonds}] \quad (5)$$

This model has greater predictive strength (R^2_{adj} = 95.2%) than the previous best predictive models, and all the variables are significant ($\alpha = 0.05$). The signs and relative magnitudes also show a good consistency with those of the previous models reported attained in this study. It is believed that this new model depicts a meaningful improvement over the previous best model (according to MUFA, DiUFA, and TriUFA), and has accounted for the prioritization and consideration of the TAG double bond distribution in a way that would have been lost by a less sequential approach.

Following this step, the residuals of this new best model were compared to the remaining possible correlatory factors, and it was determined that fatty acid composition best explained the remaining inefficiencies at that point. This factor was then incorporated into a new best model in the manner shown above, and the process was repeated. Purity was incorporated next, but its binary nature motivated model improvement by separation into two models. Endogenous vitamins were the final meaningful contributor found. As mentioned above, positional isomers, monoacylglycerols, and free fatty acid concentration were considered at each step of the model-improvement process, but failed in all cases to show efficacy for the improvement of the models.

CONCLUSIONS

Redundancy can be a major challenge in the development of predictive mathematical models – a fact exemplified greatly by the data found in this dissertation. Although not a replacement for a controlled study design that isolates controlled variables, some success in negotiating the challenges of redundant data was found by implementing a sequential statistical approach that allowed for the natural sequential prioritization of important factors. The method allowed for the additional effects of relatively minor predictive factors to be isolated and modeled in a manner that is not overwhelmed by their possible linear associations with factors that play a larger role in outcome behavior. This technique determined that - beginning with a model for oxidative stability according to the concentrations of monounsaturated fatty acids, diunsaturated fatty acids, and triunsaturated fatty acids - the model for oxidative stability could be improved according to the sequential consideration of the following factors: (1) TAG double bond distribution, (2) fatty acid composition, (3) sample purity, and (4) endogenous vitamin concentration. TAG positional isomer data, monoacylglycerol concentration, and free fatty acid concentration were also examined, but were not found to be significant factors. This technique and its results serve as the basis for the sequential improvement of oxidative stability models found in the remaining chapters of this dissertation.

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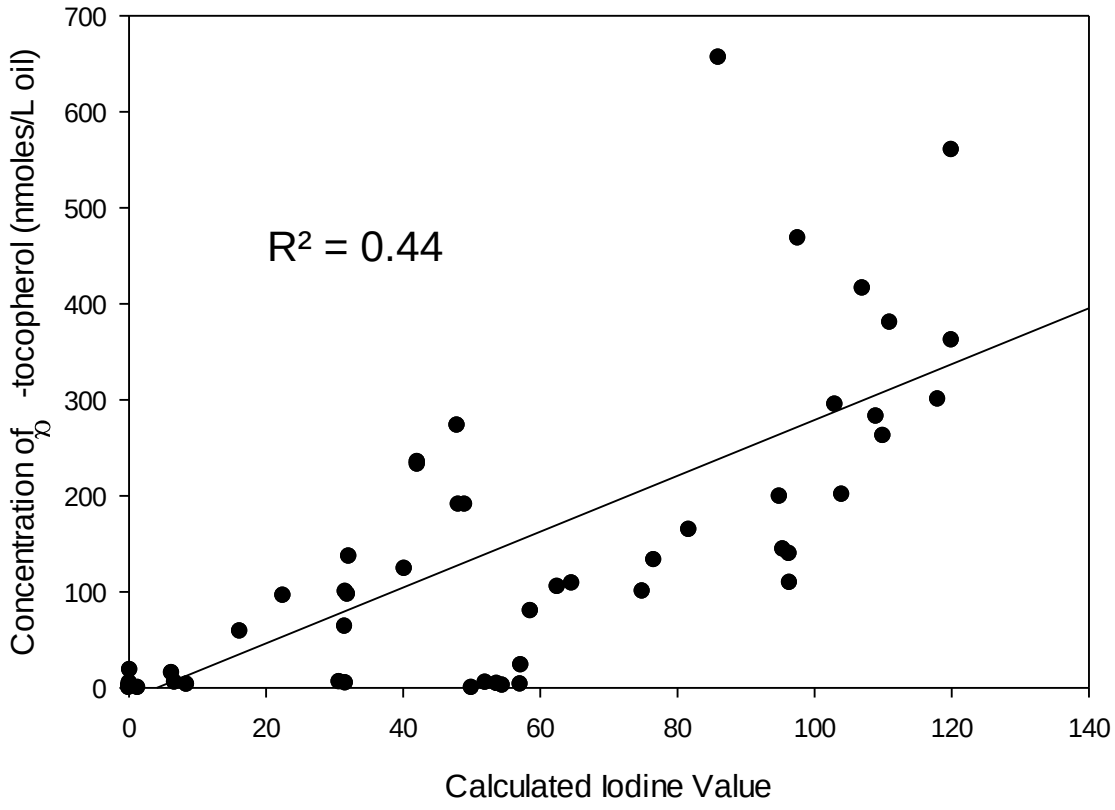

Figure 5.1 Correlation within sample set between calculated iodine value and concentration of α-tocopherol.

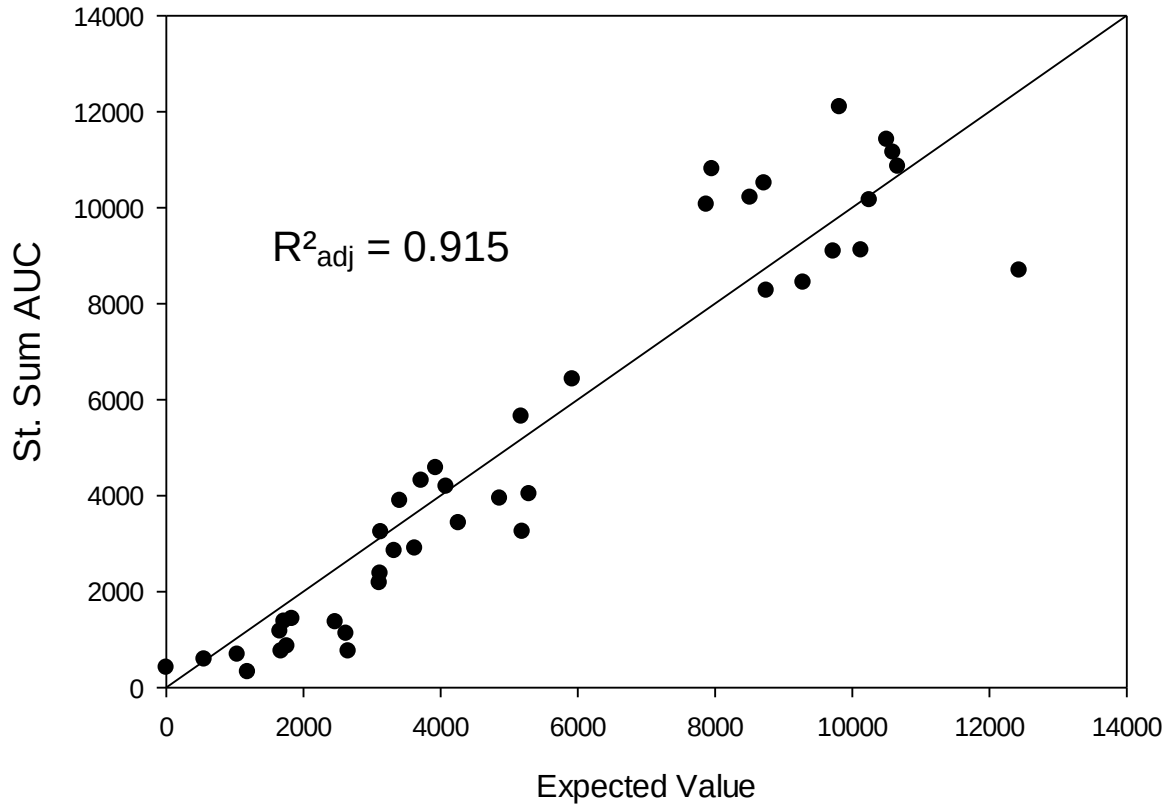

Figure 5.2 Previous best model for oxidative stability (correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA)

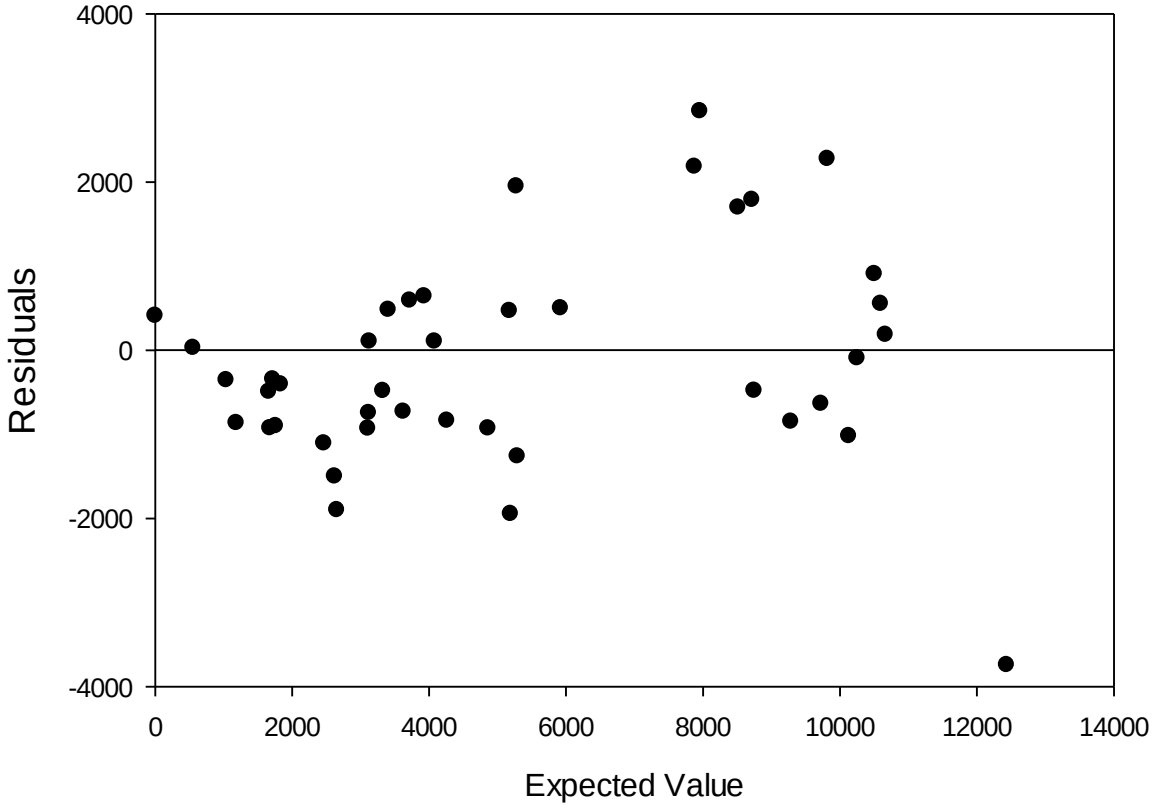

Figure 5.3 Residuals of previous best model for oxidative stability **(**correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA)

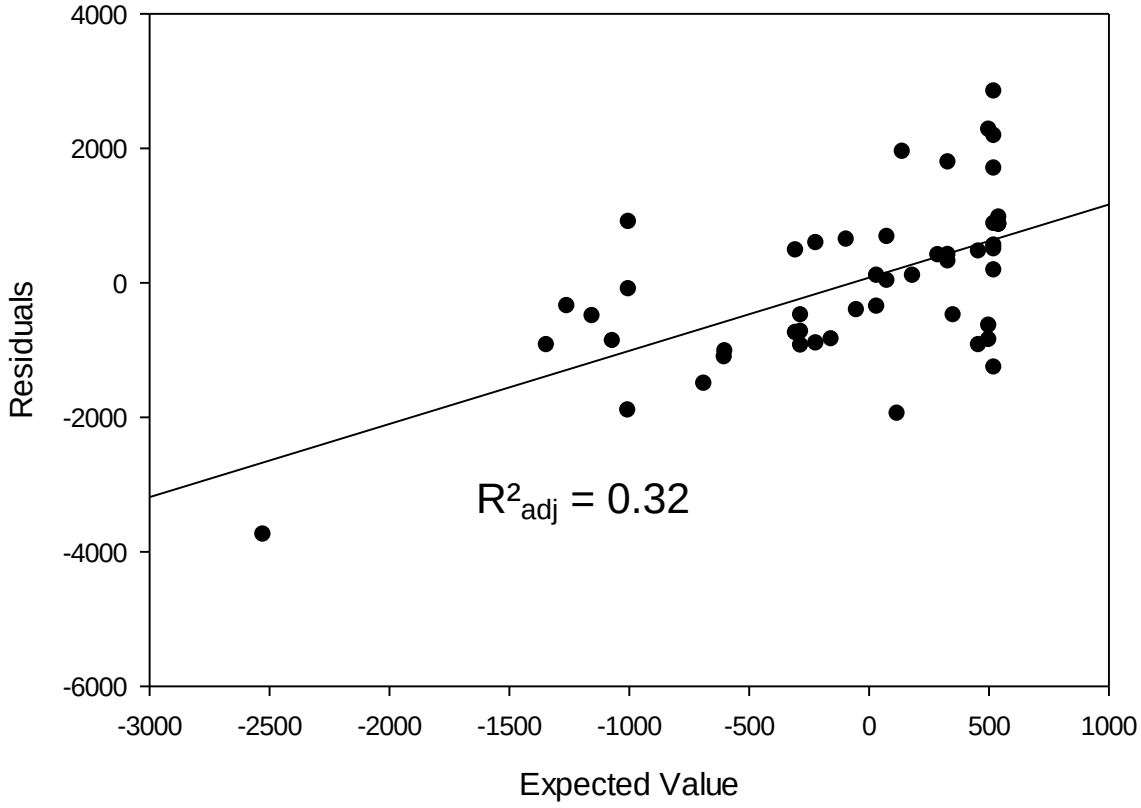

Figure 5.4 Residuals of previous best model for oxidative stability (correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA), modeled according to triacylglycerol double bond distribution)

Table 5.1 Double bond distribution among triacylglycerols (g/100 g oil)

Sample	TAG 1 double bond	TAG 2 double bonds	TAG 3 double bonds	TAG 4 double bonds	TAG 5 double bonds	TAG 6 double bonds	TAG 7 double bonds
MCTs 1	0	0	0	0	0	0	0
MCTs 2	0	0	0	0	0	0	0
Coconut Oil	0	0	0	0	0	0	0
Cocoa Butter Substitute	0	0	0	0	0	0	0
Hydrogenated Oil Blend	1	1	0	0	0	0	0
Palm Kernel Stearin Oil	10	4	1	0	0	0	0
Hydrogenated Palm Blend	10	3	1	0	0	0	0
Coconut Oil	12	3	0	0	0	0	0
Palm Kernel Oil	22	12	4	2	1	1	0
Palm Kernel Olein Oil	24	12	11	1	0	1	0
Palm Stearin Oil	36	23	9	3	1	0	0
Cocoa Butter Substitute	76	16	5	1	0	0	0
Cocoa Butter Equivalent 1	89	12	2	0	0	0	0
Palm/Coconut Mix	28	23	9	3	1	0	0
Cocoa Butter Equivalent 2	80	16	3	1	0	0	0
Cocoa Butter Equivalent 3	85	12	5	0	0	0	0
Shea Oil/Palm	54	29	10	1	0	0	0
Palm Oil 1	73	22	6	1	0	0	0
Palm Oil 2	58	30	10	2	0	0	0
Palm Oil 3	39	38	16	5	1	0	0
Palm/Coconut High Oleic	17	44	20	6	1	0	0
Palm Olein Oil 1	40	37	15	4	1	0	0
Cocoa Butter Replacer	22	46	31	6	0	0	0
Palm Oil 4	39	38	16	5	1	0	0
Palm Olein Oil 2	40	42	16	4	1	0	0
Palm Olein Oil 3	39	42	17	5	1	0	0
Palm Olein Oil 4	36	43	18	6	1	0	0
Palm/Soybean/Canola	33	33	16	7	4	4	0
Palm Olein Oil 5	30	47	20	6	1	0	0
Palm Olein Oil 6	24	50	22	7	1	0	0
Oil Mix A	20	21	18	14	9	7	0
Oil Mix B	19	22	19	13	10	6	0
Olive Oil 1	4	33	53	12	3	0	0
Olive Oil 2	4	28	55	15	4	0	0
Sunflower Oil High Oleic 1	1	21	64	9	5	5	0
Sunflower Oil High Oleic 2	1	15	64	11	7	6	0
Oil Mix C	1	12	54	17	13	7	0
Canola Oil 1	1	12	52	19	14	7	0
Corn/Canola Mix	9	17	26	24	18	11	0
Corn/Canola Mix D	10	17	26	24	18	11	0
Canola/Sunflower/Corn	1	11	43	23	17	10	0
Canola Oil 2	0	8	38	28	20	8	3
Canola Oil 3	1	8	33	28	23	9	4
Corn Oil 1	2	10	22	31	25	15	0
Corn/Canola Mix E	1	8	26	28	22	15	4
Corn Oil 2	2	10	20	30	26	17	0
Corn Oil 3	2	9	19	31	25	18	0
Corn Oil 4	1	7	16	30	28	23	0
Sunflower Oil	1	8	16	27	24	21	8
Soybean Oil	1	6	17	30	30	21	0

Table 5.2 *trans*-Fatty acids and unsaturated fatty acids longer than 18 carbons in samples (g/100 g oil)

Sample	C18:1(*trans*)	C18:2(*trans*)	C18:3(*trans*)	C22:1n-9	C22:5n-3	C24:1n-9
MCTs 1	0.00	0.00	0.00	0.00	0.00	0.00
MCTs 2	0.06	0.00	0.00	0.00	0.00	0.00
Coconut Oil	0.06	0.02	0.00	0.00	0.00	0.00
Cocoa Butter Substitute	0.17	0.00	0.00	0.00	0.00	0.00
Hydrogenated Oil Blend	0.00	0.00	0.00	0.00	0.00	0.00
Palm Kernel Stearin Oil	5.93	0.33	0.00	0.00	0.00	0.00
Hydrogenated Palm Blend	49.15	1.44	0.00	0.00	0.00	0.00
Coconut Oil	0.07	0.05	0.00	0.00	0.00	0.00
Palm Kernel Oil	0.08	0.29	0.00	0.00	0.00	0.00
Palm Kernel Olein Oil	0.15	0.23	0.00	0.00	0.00	0.00
Palm Stearin Oil	0.03	0.09	0.00	0.00	0.00	0.00
Cocoa Butter Substitute	0.06	0.18	0.00	0.00	0.00	0.00
Cocoa Butter Equivalent 1	0.09	0.06	0.00	0.00	0.00	0.00
Palm/Coconut Mix	0.11	0.15	0.00	0.00	0.00	0.00
Cocoa Butter Equivalent 2	0.04	0.34	0.00	0.00	0.00	0.03
Cocoa Butter Equivalent 3	0.00	0.07	0.00	0.00	0.00	0.00
Shea Oil/Palm	0.07	0.54	0.09	0.00	0.00	0.00
Palm Oil 1	0.04	0.15	0.18	0.07	0.00	0.00
Palm Oil 2	0.04	0.18	0.22	0.00	0.00	0.05
Palm Oil 3	0.02	0.12	0.34	0.11	0.00	0.00
Palm/Coconut High Oleic	0.08	0.20	0.00	0.00	0.00	0.00
Palm Olein Oil 1	0.03	0.21	0.27	0.07	0.00	0.05
Cocoa Butter Replacer	0.00	0.14	0.31	0.04	0.00	0.08
Palm Oil 4	0.00	0.04	0.00	0.00	0.00	0.00
Palm Olein Oil 2	0.00	0.00	0.00	0.00	0.00	0.00
Palm Olein Oil 3	0.08	0.07	0.00	0.00	0.00	0.00
Palm Olein Oil 4	0.02	0.02	0.00	0.00	0.00	0.00
Palm/Soybean/Canola	0.03	0.12	0.00	0.00	0.00	0.00
Palm Olein Oil 5	0.06	0.20	0.03	0.00	0.00	0.00
Palm Olein Oil 6	0.09	0.57	0.00	0.00	0.00	0.00
Oil Mix A	0.00	0.34	0.00	0.00	0.00	0.00
Oil Mix B	0.05	0.55	0.05	0.00	0.00	0.00
Olive Oil 1	0.04	0.47	0.05	0.00	0.00	0.00
Olive Oil 2	0.04	0.20	0.00	0.00	0.00	0.00
Sunflower Oil High Oleic 1	0.06	0.11	0.07	0.00	0.00	0.00
Sunflower Oil High Oleic 2	0.00	0.08	0.00	0.00	0.00	0.00
Oil Mix C	0.00	0.29	0.04	0.00	0.00	0.00
Canola Oil 1	0.00	0.30	0.00	0.00	0.00	0.00
Corn/Canola Mix	0.07	0.39	0.04	0.00	0.00	0.00
Corn/Canola Mix D	0.03	0.14	0.06	0.00	0.00	0.00
Canola/Sunflower/Corn	0.04	0.06	0.00	0.00	0.03	0.00
Canola Oil 2	0.00	0.33	0.00	0.00	0.04	0.00
Canola Oil 3	0.04	0.28	0.49	0.00	0.00	0.00
Corn Oil 1	0.00	0.29	0.09	0.00	0.00	0.00
Corn/Canola Mix E	0.00	0.11	0.52	0.10	0.00	0.13
Corn Oil 2	0.02	0.08	0.42	0.08	0.00	0.00
Corn Oil 3	0.02	0.13	0.35	0.11	0.00	0.00
Corn Oil 4	0.00	0.14	0.06	0.00	0.00	0.00
Sunflower Oil	0.00	0.23	0.00	0.00	0.00	0.00
Soybean Oil	0.00	0.10	0.06	0.00	0.00	0.00

Table 5.3 Sample set according to classification as pure or blended

Blended Samples		Pure Samples	
Sample Name	**CIV**	**Sample Name**	**CIV**
MCTs 1	0	Coconut Oil	0.1
MCTs 2	0	Palm Kernel Stearin Oil	6.25
Cocoa Butter Substitute	0.15	Coconut Oil	8.45
Hydrogenated Oil Blend	1.33	Palm Kernel Oil	16.2
Hydrogenated Palm Blend	6.71	Palm Kernel Olein Oil	22.5
Cocoa Butter Substitute	31.5	Palm Stearin Oil	30.7
Cocoa Butter Equivalent 1	31.6	Palm Oil 3	47.8
Palm/Coconut Mix	31.6	Palm Olein Oil 1	48.1
Cocoa Butter Equivalent 2	31.9	Palm Oil 4	50
Cocoa Butter Equivalent 3	32.1	Palm Olein Oil 2	52
Shea Oil/Palm Blend	40.2	Palm Olein Oil 3	53.7
Palm Oil 1	42.1	Palm Olein Oil 4	54.5
Palm Oil 2	42.1	Palm Olein Oil 5	57.2
Palm/Coconut High Oleic	47.9	Palm Olein Oil 6	58.6
Cocoa Butter Replacer	49	Olive Oil 1	74.9
Palm/Soybean/Canola	57.1	Olive Oil 2	76.6
Oil Mix A	62.5	Sunflower Oil High Oleic 1	81.7
Oil Mix B	64.6	Sunflower Oil High Oleic 2	86
Oil Mix C	94.9	Canola Oil 1	95.4
Corn/Canola Mix	96.3	Canola Oil 2	103.2
Corn/Canola Mix D	96.4	Canola Oil 3	104
Canola/Sunflower/Corn	97.6	Corn Oil 1	107
Corn/Canola Mix E	109	Corn Oil 2	110
		Corn Oil 3	111
		Corn Oil 4	118
		Sunflower Oil	120
		Soybean Oil	120

Table 5.4 Endogenous vitamin concentrations in samples (nmoles/L oil)

Sample	β-Carotene	Vitamin K	Retinyl acetate	Tocopherols				Tocotrienols			
				α	β	γ	σ	α	β	γ	σ
MCTs 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MCTs 2	0.0	0.0	0.0	1.9	0.0	1.9	0.0	0.0	0.0	0.0	0.0
Coconut Oil	0.0	0.0	0.0	5.1	0.0	4.0	1.3	0.0	0.0	0.0	0.0
Cocoa Butter Substitute	0.0	0.0	0.0	18.6	1.8	8.8	2.9	1.3	0.0	2.4	0.0
Hydrogenated Oil Blend	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palm Kernel Stearin Oil	2.6	0.0	0.0	15.3	1.9	58.6	18.6	0.0	0.0	0.0	3.1
Hydrogenated Palm Blend	0.0	0.0	0.0	5.6	1.6	32.4	11.0	0.0	0.0	0.0	0.0
Coconut Oil	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	5.3	2.7
Palm Kernel Oil	3.4	0.0	0.0	58.7	0.0	7.8	2.9	51.3	10.3	95.6	17.0
Palm Kernel Olein Oil	7.1	0.0	0.0	95.9	0.0	7.6	0.0	68.0	12.2	137.4	27.8
Palm Stearin Oil	0.0	0.0	0.0	6.0	0.0	0.0	0.0	5.9	0.0	17.3	5.1
Cocoa Butter Substitute	3.5	0.0	0.0	63.7	0.0	0.0	0.0	56.0	11.1	124.4	23.7
Cocoa Butter Equivalent 1	0.0	0.0	0.0	4.7	0.0	0.0	0.0	0.0	0.0	4.5	2.2
Palm/Coconut Mix	5.0	0.0	0.0	99.9	0.0	5.4	0.0	94.4	16.0	171.2	32.2
Cocoa Butter Equivalent 2	6.8	0.0	0.0	97.1	0.0	2.6	0.0	86.8	0.0	145.5	36.5
Cocoa Butter Equivalent 3	5.8	0.0	0.0	136.7	3.9	256.4	32.7	78.4	15.4	150.4	35.5
Shea Oil/Palm	5.2	0.0	0.0	123.9	7.6	388.5	147.2	79.9	14.6	154.5	28.7
Palm Oil 1	4.2	0.0	0.0	232.5	4.6	102.4	2.1	67.2	12.3	106.4	20.6
Palm Oil 2	2.6	1.1	0.0	235.2	5.8	648.3	21.3	32.5	9.4	73.1	15.9
Palm Oil 3	0.0	1.1	0.0	418.2	14.8	212.3	4.1	0.0	0.0	0.0	0.0
Palm/Coconut High Oleic	0.0	1.4	0.0	273.2	6.1	345.3	8.8	2.6	0.0	3.1	0.0
Palm Olein Oil 1	2.1	0.0	0.0	190.8	4.1	565.2	17.5	31.6	0.0	60.3	15.3
Cocoa Butter Replacer	0.0	1.3	0.0	190.7	7.3	912.2	53.7	5.2	0.0	14.5	1.1
Palm Oil 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palm Olein Oil 2	0.0	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palm Olein Oil 3	0.0	0.0	0.0	4.1	0.0	0.0	0.0	26.8	7.3	12.1	2.5
Palm Olein Oil 4	0.0	0.0	0.0	2.4	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Palm/Soybean/Canola	0.0	0.0	2.0	3.6	0.0	0.0	0.0	16.3	0.0	4.2	0.0
Palm Olein Oil 5	8.8	0.0	0.0	23.6	1.5	2.7	0.0	19.0	8.4	83.0	17.7
Palm Olein Oil 6	3.3	0.0	0.0	80.0	0.0	1.8	0.0	83.4	0.0	114.7	25.6
Oil Mix A	7.9	0.0	0.0	105.1	0.0	0.0	0.0	129.4	22.7	245.9	50.5
Oil Mix B	5.4	0.0	0.0	108.7	1.4	0.0	0.0	86.0	14.6	179.1	29.2
Olive Oil 1	6.9	0.0	0.0	100.4	0.0	0.0	0.0	104.9	16.7	199.4	33.6
Olive Oil 2	17.1	0.0	0.0	133.0	0.0	0.0	0.0	162.9	23.9	337.9	50.4
Sunflower Oil High Oleic 1	0.0	0.0	0.0	164.6	1.9	13.0	0.0	0.0	0.0	0.0	0.0
Sunflower Oil High Oleic 2	0.0	0.0	0.0	656.2	17.9	2.8	0.0	0.0	0.0	0.0	0.0
Oil Mix C	10.9	0.0	0.0	199.1	3.2	70.6	27.1	150.4	24.6	319.2	59.1
Canola Oil 1	7.3	0.0	0.0	144.2	0.0	0.0	0.0	136.2	20.8	276.2	47.4
Corn/Canola Mix	7.3	0.0	0.0	139.6	0.0	1.9	0.0	141.4	20.6	234.1	50.5
Corn/Canola Mix D	0.0	0.0	0.0	109.3	0.0	6.1	0.0	0.0	0.0	0.0	0.0
Canola/Sunflower/Corn	0.0	0.0	0.0	468.2	10.4	5.2	1.3	0.0	0.0	0.0	0.0
Canola Oil 2	0.0	0.0	0.0	294.9	7.4	836.7	19.5	11.9	0.0	19.2	1.8
Canola Oil 3	0.0	2.1	0.0	201.1	29.4	767.5	329.6	0.0	0.0	3.0	1.3
Corn Oil 1	0.0	0.0	0.0	416.0	15.3	768.7	22.2	10.2	0.0	10.8	0.0
Corn/Canola Mix E	0.0	1.7	0.0	282.5	3.5	419.1	7.0	0.0	0.0	0.0	0.0
Corn Oil 2	0.0	1.6	0.0	262.3	3.4	391.0	8.1	0.0	0.0	2.3	0.0
Corn Oil 3	0.0	1.0	0.0	380.3	12.0	174.6	3.1	0.0	0.0	0.0	0.0
Corn Oil 4	0.0	0.0	0.0	300.4	14.0	10.6	1141.5	0.0	23.0	36.4	1.5
Sunflower Oil	0.0	0.0	0.0	560.1	0.0	18.7	11.4	0.0	0.0	4.4	0.0
Soybean Oil	0.0	0.0	0.0	362.0	34.9	14.2	1208.7	0.0	58.0	45.2	2.7

Table 5.5 Correlations of possible factor considerations to unexplained variance of previous best model for oxidative stability (made according to concentrations of MUFA, DiUFA, and TriUFA)

Factor	**Correlation Strength ($R^2_{Adjusted}$)**
Triacylglycerol Double Bond Distribution	33.8%
Fatty Acid Composition	7.5%
Sample Purity	0%
Vitamin Concentration	12.7%

CHAPTER 6

MODELING THE EFFECT OF DOUBLE BOND DISTRIBUTION WITHIN TRIACYLGLYCEROLS ON THE ACCUMULATION OF LIPID AUTOXIDATION PRODUCTS

INTRODUCTION

The oxidative deterioration of lipids in food products can affect food safety, nutrition, texture, color, as well as result in the release of aromatic volatile compounds responsible for undesirable or "rancid" flavors associated with poor food quality (Gray, 1978; Ajuyah et al., 1993; Morales et al., 1997). Lipid oxidation is in many cases the limiting factor in the shelf life of food products, and thus, is one of the key elements that require consideration in a product's design, formulation, processing, packaging, and storage (Chaiyasit et al., 2007).

Many contributing factors to lipid oxidation have been cited including: fatty acid composition (particularly the degree of unsaturation), storage temperature, processing methods, and the concentrations of oxygen, free fatty acids, pro-oxidants, and antioxidants. However, the observed effects of these factors in scientific study have been highly inconsistent – which has meant that means to accurately predict and/or control lipid oxidation have proved elusive (Lea and Hawke, 1951; Min and Boff, 2002).

Studies have demonstrated an observed effect to oxidative stability attributable to the distribution of double bonds within fatty acids (the occurrence of multiple double bonds on a single fatty acid appear to impair stability to a greater extent than when double bonds occur on separate fatty acids; see chapter 4 of this dissertation for more detail). One of the mechanisms of

this effect is due to the heightened reactivity of bis-allylic sites, but additional observed synergies are numerous and are still under investigation. It is reasonable to hypothesize that the initiation of lipid autooxidation at one double bond may impair the energetic stability of double bonds that are within close physical proximity.

One possible factor in regards to lipid oxidative stability that has received little attention in scientific literature is the distribution of double bonds within the triacylglycerols (TAG) of a lipid sample. Due to the observed effect of double bond distribution within fatty acids, the distribution within TAG may be of possible importance to oxidative stability.

This study builds upon the data and modeling efforts of chapter 3 (which developed a quantitative definition for oxidative stability) and chapter 4, which modeled oxidative stability according to concentrations of monounsaturated fatty acids (MUFA), diunsaturated fatty acids (DiUFA), and triunsaturated fatty acids (TriUFA). This previous best model was depicted graphically in **Figure 4.2**, and is as follows (1):

$$\text{St. Sum AUC} = -618 + 53.2[\text{MUFA}] + 167[\text{DiUFA}] + 635\,[\text{TriUFA}] \quad (1)$$

This model had a correlation of $R^2_{adj} = 91.5\%$. The objective of this paper is to incorporate the additional consideration of regioisomeric TAG data. The goal is to discern the importance of this factor, determine its specific effects, and to utilize it as a possible means to produce improved predictive models of oxidative stability.

MATERIALS AND METHODS

Oxidation data

Acquisition of oxidation data used for this chapter is described in chapter 3 of this dissertation.

Standardized Summation of Area under the Curve

For a comprehensive quantification of oxidative stability within samples, the "Standardization Summation of Area under the Curve" (St. Sum AUC) term was calculated from the curves of the four oxidative product assays. This derived term is proposed to be a meaningful and comprehensive quantitative summary of an oil or fat's oxidative stability. The derivation of this term (as well as the justification for its use) can be found in chapter 3 of this dissertation. The AUC values were computed using integral calculus computations within Sigmaplot 12.0 (Systat Software, San Jose, CA), and the St. Sum AUC values were calculated according to the following equation (2):

$$\text{St. Sum AUC} = [PV_{AUC} + (6.31)CDT_{AUC} + (2.60)TBARS_{AUC} + (2.87)p\text{-}AnV_{AUC}]/4 \quad (2)$$

Regioisomeric Distribution of Fatty Acids in Triacylglycerols

Regioisomeric distribution of fatty acids in TAG was determined by hybrid mass spectrometry according to the methodology described by Nagy et al. (2012). For each sample, this data was used to calculate the respective concentrations of TAG with one, two, three, four, five, six, and seven double bonds (there were no substantial occurrences of TAG containing more than seven double bonds).

Model Building by Multiple Linear Regression Techniques

The method for factor selection and model-building was performed as discussed in chapter 5.

Determination of Specific Relative Effects of Variables

The coefficients in predictive models can provide meaningful information regarding the relative effects of variables. However, this information is often not effectively conveyed by a single choice of a good model. For example, certain variables may be eliminated from models for the sake of simplicity, which results in the loss of information regarding the effect that variable may exhibit upon the outcome.

Therefore, this study produces seven different models for the single purpose of quantifying the effects indicated by coefficients. Using SAS software (as described above), seven models were created in which each of the seven possible variables for this factor were inputted into the previous best model. These values were standardized according to magnitude (i.e. to make the lowest value equal to +/- 1.0). The reported values are to be interpreted as approximate indicators of the relative effects (upon oxidation outcome) of the variables within the considered factor.

RESULTS AND DISCUSSION

Regioisomeric Distribution of Fatty Acids in Triacylglycerols

Table 6.1 shows the concentrations of TAG with one, two, three, four, five, six, and seven double bonds for each of the 50 samples. This table shows the samples in order of ascending unsaturation. Samples that are more unsaturated of course show higher concentrations of more

highly unsaturated TAG, but the table shows that the specific distributions of double bonds across TAG do not follow a clear systematic pattern as unsaturation increases.

Model Building by Multiple Linear Regression Techniques

Figures 6.1, **6.2**, **6.3**, and **6.4** depict the sequential steps of the improvement to our previous best model for oxidative stability. **Figure 6.1** shows our previous best model, which modeled the St. Sum AUC according to the concentrations, of MUFA, DiUFA, and TriUFA. **Figure 6.2** shows the oxidative behavior that is unexplained by this model. **Figure 6.3** depicts the result of efforts to model this unexplained oxidative behavior by consideration of TAG double bond distribution. The two variables used in this model (concentration of TAG with one double bond, and concentration of TAG with seven double bonds) are both significant ($\alpha = 0.05$), and explain 32% of the previously unexplained variance. The R^2_{adj} of 32% may be fairly low according to some predictive standards, but here it represents a substantial improvement to a model that had already accounted for approximately 91% of the behavior of the St. Sum AUC outcome variable. **Figure 6.4** shows the new model that incorporates the two new variables into the previous best model. This model is as follows (3):

$$\text{St. Sum AUC} = 58 + 47.9[\text{MUFA}] + 168[\text{DiUFA}] + 883[\text{TriUFA}] - 19.3[\text{TAG w/ 1 double bond}] - 759[\text{TAG w/ 7 double bonds}] \quad (3)$$

The correlation strength of the model considering TAG double bonds distribution (R^2_{adj} = 95.2%) is a substantial improvement over that observed when not considering this factor (R^2_{adj} = 91.5%). All variables in the model are significant ($\alpha = 0.05$), and combined present a very strong predictive relationship with St. Sum AUC. These results suggest that double bond distribution

amongst TAG can serve as an important and significant consideration for the prediction of oxidative stability within fats and oils.

Determination of Specific Relative Effects of Variables

Figure 6.5 shows the standardized coefficients of each of the seven possible variables for the factor of TAG double bonds distribution. The values of these coefficents represent an inverse relationship with oxidative stability (as defined quantitatively by our St. Sum AUC term). The results suggest the concentrations of TAG containing one, two, and seven double bonds were positively associated with stability, and the concentrations of TAG containing three, four, five, and six double bonds were negatively associated with stability. The correlatory effect upon observed stability is of the greatest magnitude in the case of TAG with seven double bonds.

It is important to bear in mind that these effects are independent of sample unsaturation. So, for example, although the presence of a TAG containing seven double bonds will likely actually impair sample stability due to its high degree of unsaturation, these results suggest that the occurrence of seven double bonds on a single TAG is associated with a more stable system than if those seven double bonds were distributed across more than one TAG.

To the author's knowledge, there is no published literature examining this specific possible effect, so the effect observed here is neither in agreement or disagreement with expectations. Given the previously observed synergistic oxidative effect associated with the combined presence of double bonds on a single fatty acid (see chapter 4 of this dissertation), it is reasonable to speculate similar physical phenomena may be occurring here (particularly with the pro-oxidative effect of combining three, four, five, or six double bonds on a single TAG, as opposed to one or two).

One hypothesized physical explanation for this observed effect is that the initiation of oxidation at one double bond may lead to pro-oxidative deterioration of energy stability for nearby double bonds. Another hypothesis is that the initiation of oxidative reactions may produce pro-oxidative compounds, which in turn are more likely to initiate additional oxidative reactions if in close proximity to other possible reaction sites (i.e. double bonds or bis-allylic sites). The pro-oxidative effects of hydroperoxides, for example, could very conceivably be more deleterious to oxidative stability if the compounds are formed within immediate vicinity of sources for possible oxidative initiation reactions (Chaiyasit et al., 2007; Kim et al., 2007, Morita and Fujimaki, 1973; Morita et al., 1976; Morita and Tokita 2006). The results of this study also suggest that perhaps this a limiting factor upon oxidation occurs once more than six double bonds share a single TAG. This may be a result of steric hindrance.

The results here indicate that the possible importance of this factor likely deserves further scientific examination.

CONCLUSIONS

It was determined that the distribution of double bonds within TAG was a statistically significant factor for the prediction of lipid autoxidative stability. The consideration of this factor combined with the consideration of the concentrations of MUFA, DiUFA, and TriUFA demonstrated a very strong predictive relationship (R^2_{adj} = 95.2%) with the measure of exhibited accumulation of oxidation products within samples of edible oils and fats. Independent of sample unsaturation, the concentrations of TAG containing one, two, and seven double bonds were positively associated with stability, and the concentrations of TAG containing three, four, five, and six double bonds were negatively associated with stability. The results of this study

suggest that the distribution of double bonds within TAG warrants additional investigation as a means of predicting, and possibly optimizing, oxidative stability within edible fats and oils.

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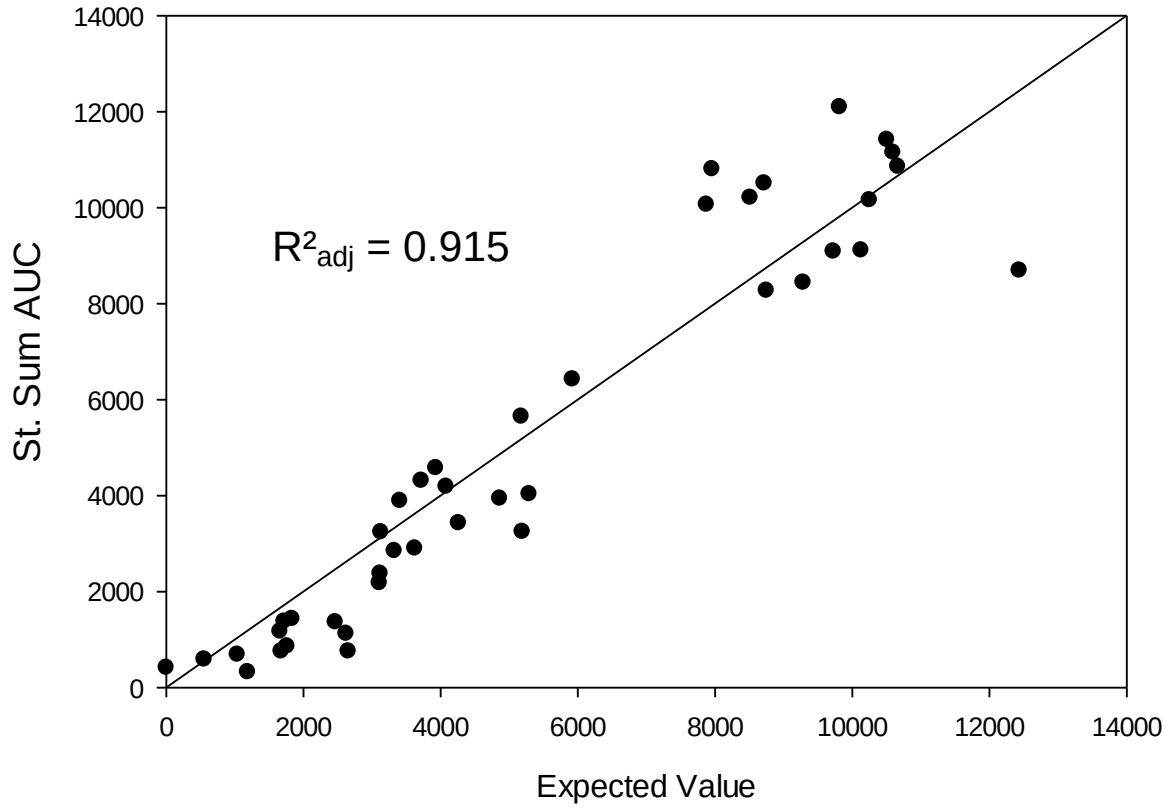

Figure 6.1 Previous best model for oxidative stability (correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA)

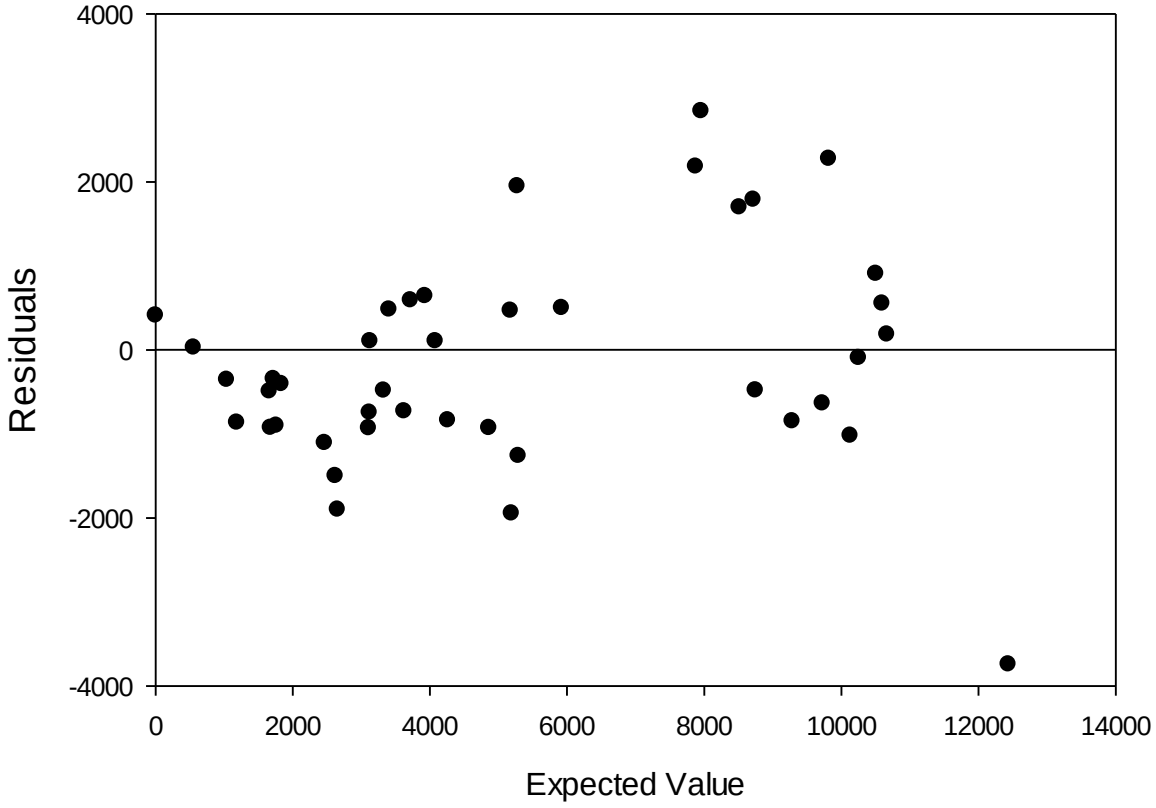

Figure 6.2 Residuals of previous best model for oxidative stability **(**correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA)

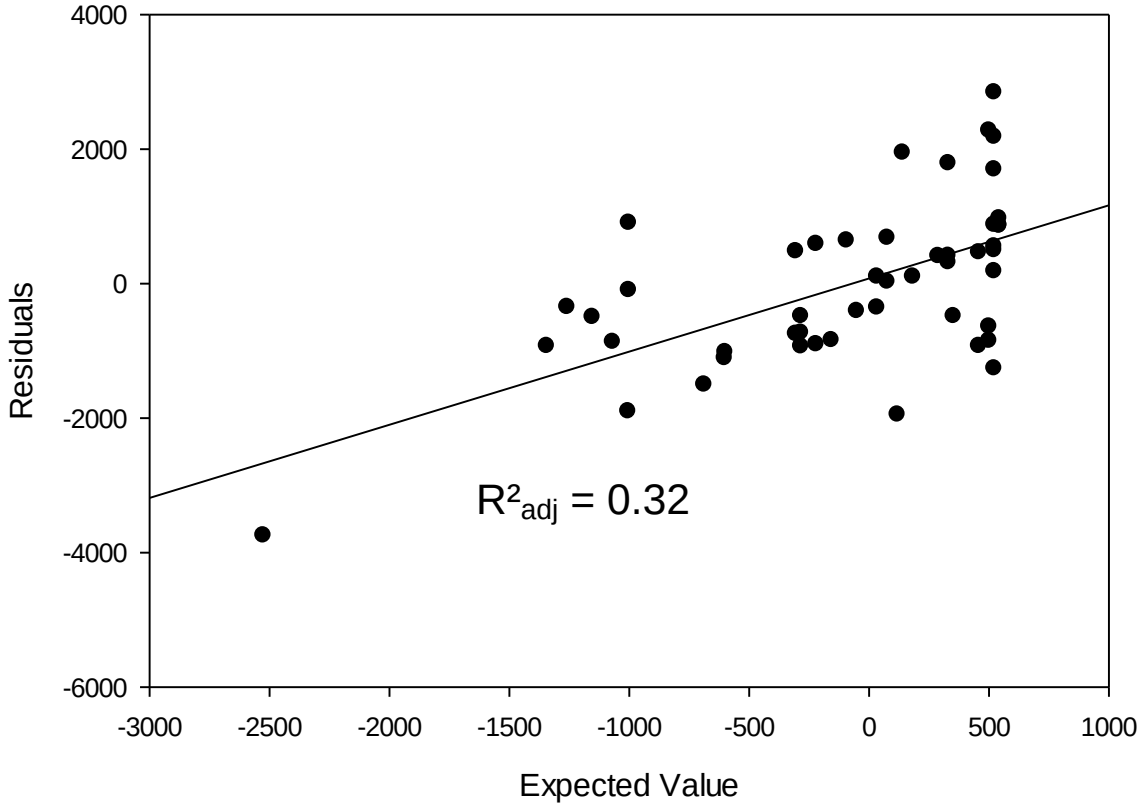

Figure 6.3 Residuals of previous best model for oxidative stability (according to concentrations of MUFA, DiUFA, and TriUFA), modeled according to triacylglycerol double bond distribution

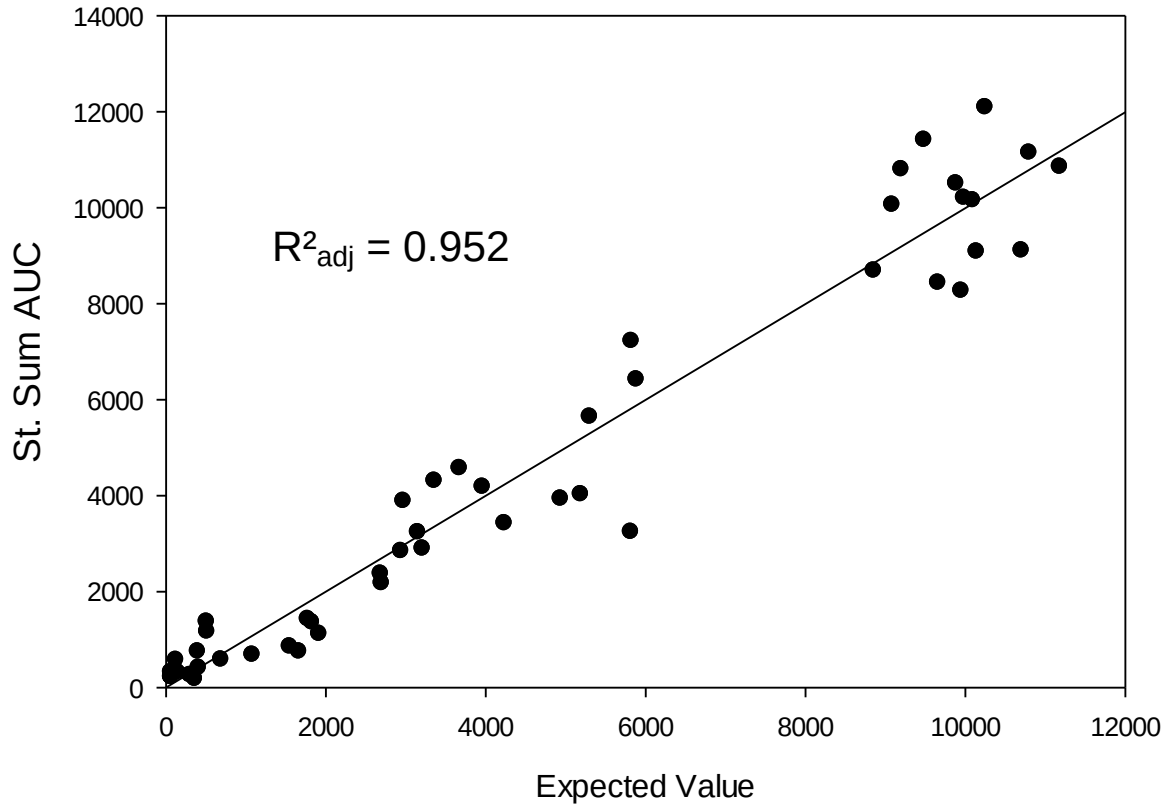

Figure 6.4 New model for the prediction of oxidative stability (correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA, and triacylglycerol double bond distribution)

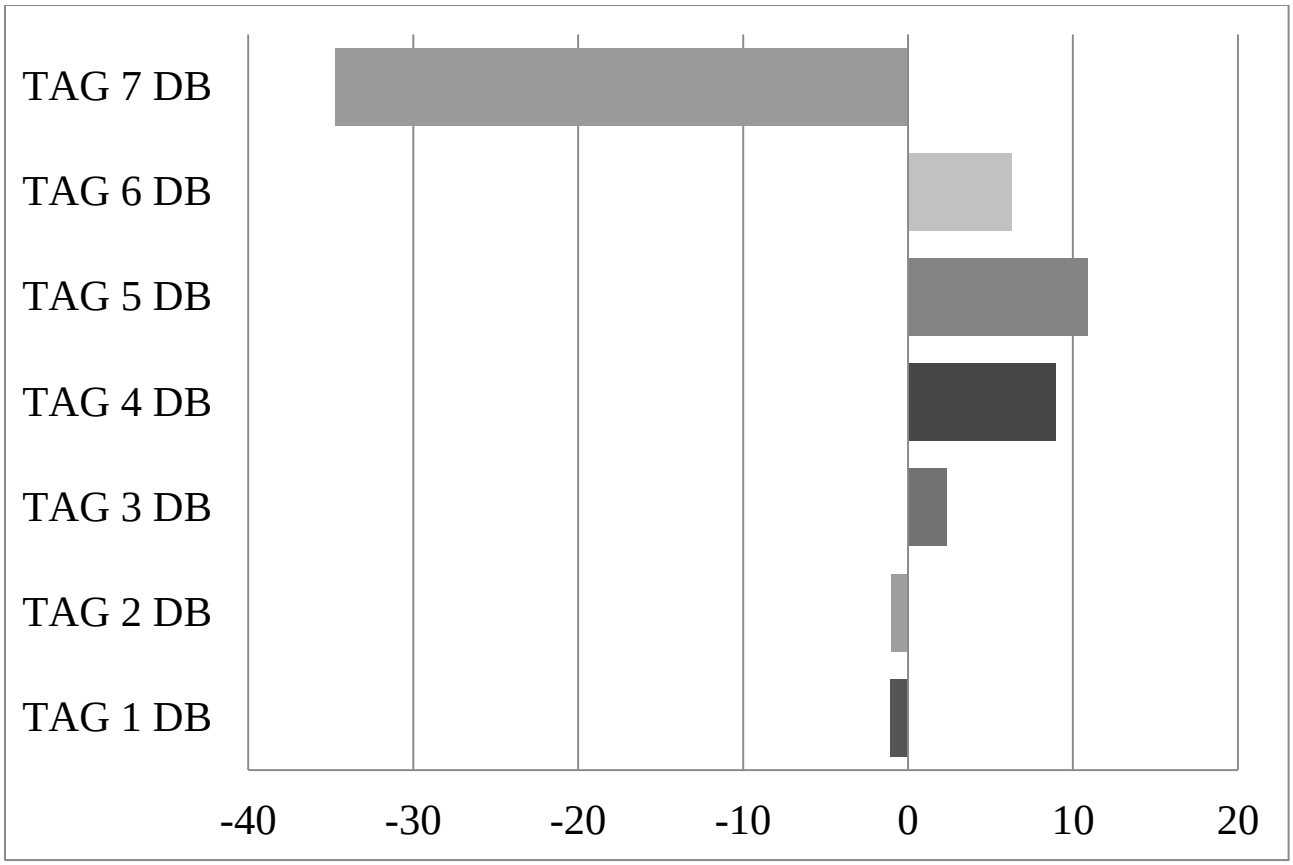

Figure 6.5 Relative observed correlatory effect of variables within the factor of triacylglycerol double bond distribution upon observed oxidation (standardized coefficients of 7 models)

Table 6.1 Double bond distribution among triacylglycerols (g/100 g oil)

Sample	TAG 1 Double Bonds	TAG 2 Double Bonds	TAG 3 Double Bonds	TAG 4 Double Bonds	TAG 5 Double Bonds	TAG 6 Double Bonds	TAG 7 Double Bonds
MCTs 1	0	0	0	0	0	0	0
MCTs 2	0	0	0	0	0	0	0
Coconut Oil	0	0	0	0	0	0	0
Cocoa Butter Substitute	0	0	0	0	0	0	0
Hydrogenated Oil Blend	1	1	0	0	0	0	0
Palm Kernel Stearin Oil	10	4	1	0	0	0	0
Hydrogenated Palm Blend	10	3	1	0	0	0	0
Coconut Oil	12	3	0	0	0	0	0
Palm Kernel Oil	22	12	4	2	1	1	0
Palm Kernel Olein Oil	24	12	11	1	0	1	0
Palm Stearin Oil	36	23	9	3	1	0	0
Cocoa Butter Substitute	76	16	5	1	0	0	0
Cocoa Butter Equivalent 1	89	12	2	0	0	0	0
Palm/Coconut Mix	28	23	9	3	1	0	0
Cocoa Butter Equivalent 2	80	16	3	1	0	0	0
Cocoa Butter Equivalent 3	85	12	5	0	0	0	0
Shea Oil/Palm	54	29	10	1	0	0	0
Palm Oil	73	22	6	1	0	0	0
Palm Oil	58	30	10	2	0	0	0
Palm Oil 3	39	38	16	5	1	0	0
Palm/Coconut High Oleic	17	44	20	6	1	0	0
Palm Olein Oil 1	40	37	15	4	1	0	0
Cocoa Butter Replacer	22	46	31	6	0	0	0
Palm Oil 4	39	38	16	5	1	0	0
Palm Olein Oil 2	40	42	16	4	1	0	0
Palm Olein Oil 3	39	42	17	5	1	0	0
Palm Olein Oil 4	36	43	18	6	1	0	0
Palm/Soybean/Canola	33	33	16	7	4	4	0
Palm Olein Oil 5	30	47	20	6	1	0	0
Palm Olein Oil 6	24	50	22	7	1	0	0
Oil Mix A	20	21	18	14	9	7	0
Oil Mix B	19	22	19	13	10	6	0
Olive Oil 1	4	33	53	12	3	0	0
Olive Oil 2	4	28	55	15	4	0	0
Sunflower Oil High Oleic 1	1	21	64	9	5	5	0
Sunflower Oil High Oleic 2	1	15	64	11	7	6	0
Oil Mix C	1	12	54	17	13	7	0
Canola Oil 1	1	12	52	19	14	7	0
Corn/Canola Mix	9	17	26	24	18	11	0
Corn/Canola Mix D	10	17	26	24	18	11	0
Canola/Sunflower/Corn	1	11	43	23	17	10	0
Canola Oil 2	0	8	38	28	20	8	3
Canola Oil 3	1	8	33	28	23	9	4
Corn Oil 1	2	10	22	31	25	15	0
Corn/Canola Mix E	1	8	26	28	22	15	4
Corn Oil 2	2	10	20	30	26	17	0
Corn Oil 3	2	9	19	31	25	18	0
Corn Oil 4	1	7	16	30	28	23	0
Sunflower Oil	1	8	16	27	24	21	8
Soybean Oil	1	6	17	30	30	21	0

CHAPTER 7

MODELING THE EFFECTS OF TRANS-FATTY ACIDS, UNSATURATED FATTY ACIDS GREATER THAN 18 CARBONS IN LENGTH, SAMPLE PURITY, AND ENDOGENOUS VITAMINS ON THE ACCUMULATION OF LIPID AUTOXIDATION PRODUCTS

INTRODUCTION

The oxidative deterioration of lipids in food products can affect food safety, nutrition, texture, color, as well as result in the release of aromatic volatile compounds responsible for undesirable or "rancid" flavors associated with poor food quality (Gray, 1978; Ajuyah et al., 1993; Morales et al., 1997). Lipid oxidation is in many cases the limiting factor in the shelf life of food products, and thus, is one of the key elements that require consideration in a product's design, formulation, processing, packaging, and storage (Chaiyasit et al., 2007).

Many contributing factors to lipid oxidation have been cited including: fatty acid composition (particularly the degree of unsaturation), storage temperature, processing methods, and the concentrations of oxygen, free fatty acids, pro-oxidants, and antioxidants. However, the observed effects of these factors in scientific study have been highly inconsistent – which has meant that means to accurately predict and/or control lipid oxidation have proved elusive (Lea and Hawke, 1951; Min and Boff, 2002).

Studies have demonstrated an observed effect to oxidative stability attributable to the distribution of double bonds within fatty acids (the occurrence of multiple double bonds on a single fatty acid appear to impair stability to a greater extent than when double bonds occur on separate fatty acids; see chapter 4 of this dissertation for more detail), and a similar effect has

been observed for the distribution of double bonds within triacylglycerols (TAG; see chapter 6). One of the mechanisms of this effect is due to the heightened reactivity of bis-allylic sites, but additional observed synergies are numerous and are still under investigation. It is reasonable to hypothesize that the initiation of lipid autooxidation at one double bond may impair the energetic stability of double bonds that are within close physical proximity.

Given the possible role that physical proximity of double bonds to one another may have upon oxidative stability, it is also feasible that the length of the fatty acids containing double bonds may affect stability. Overwhelmingly, the majority of unsaturated fatty acids in our modern dietary sources are 18 carbons in length, and the isolation of the possible effect of fatty acids being of greater carbon length than this has not received much investigation. Many previous scientific investigations into the effects of individual fatty acids upon oxidation have done so in a manner that is usually not independent of the unsaturation of fatty acids. For example, the long-chain and highly unsaturated fatty acids such as eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) have been shown to impair oxidative stability, but this behavior is unsurprising due to their high number of reaction sites (Arab-Tehrany et al., 2012). One very interesting recent study, however, did find improvements in observed oxidative stability with both the reduction in carbon-length of unsaturated fatty acids and also the shift of double bonds towards the ester region of the fatty acids (Moser, 2009). Both of these results indicate that some possible alleviation of steric hindrance on autoxidation reactions may impair a fatty acid's oxidative stability, but further investigation has been needed.

More thoroughly studied has been the role of *trans*-fatty acids (TFA) upon oxidative stability. These have been shown repeatedly to associate with greater oxidative stability in a system than their *cis* counterparts (Moser, 2009, Tallman et al., 2004). This occurrence is

typically attributed to the increased rigidity of the *trans* forms of fatty acids, which again evidences the possible importance of steric hindrances and physical location factors upon oxidative stability.

The blending of vegetable oils and fats has become a common technique within the food industry as a means of modifying the physicochemical characteristics of vegetable oils (Anwar et al., 2007). The combination of lipids from multiple sources allows for the specific properties and compositions of fats to be optimized without the use of hydrogenation (Ramadan and Wahdan, 2012). Although the oxidative stability of such blends has been previously investigated in literature, the direct consequence of this technique upon oxidative stability has not been clearly isolated. For example, several studies show that blending can affect the stability of an oil (e.g. (Ramadan and Wahdan, 2012, Mariod et al., 2005), but this is attributed to the characteristics of the added oil (e.g. antioxidant concentrations, degree of unsaturation, etc.), rather than a direct result of the production of a blend. There is an observed phenomenon, known sometimes as "nature's wisdom," that single-source food products tend to be of fairly optimized stabilities. This has been observed specifically for the oxidation rates of edible oils (Kamal-Eldin, 2006). It is feasible the production of blended oils may impair this optimized state and directly affect stability.

Vitamins A, E, and K are fat-soluble compounds that occur naturally in varying quantities within edible oils and fats. Vitamin A is a somewhat broad classification that includes β-carotene and retinyl acetate. Vitamin E includes numerous species of tocopherols and tocotrienols. Each of these fat-soluble vitamins has demonstrated antioxidative effect, and all are generally considered antioxidants (Carocho and Ferreira, 2013). The classification of "antioxidant", however, represents perhaps an oversimplification, as the observed effects in real

systems have been notably inconsistent – ranging from antioxidative actions to even pro-oxidative effects (Carocho and Ferreira, 2013). Specific investigations into this phenomenon have suggested this contradiction effect to be concentration-dependent - with antioxidative action deteriorating with increasing concentrations, and pro-oxidative actions eventually occurring with continued increasing concentration (Kamal-Eldin, 2006). Additionally, there are noted synergies in antioxidative action that can occur between species of natural antioxidants (e.g. a synergy between carotenoids and tocopherols) which are susceptible to similar concentration-dependent variations in effect (Schroeder, 2006).

This study builds upon the data and modeling efforts of chapter 3 (which developed a quantitative definition for oxidative stability) and chapters 4 and 6, which modeled oxidative stability according to concentrations of monounsaturated fatty acids (MUFA), diunsaturated fatty acids (DiUFA), triunsaturated fatty acids (TriUFA), and TAG double bonds. This previous best model was depicted graphically in **Figure 5.4**, and is as follows (1):

$$\text{St. Sum AUC} = 58 + 47.9[\text{MUFA}] + 168[\text{DiUFA}] + 883[\text{TriUFA}] - 19.3[\text{TAG w/ 1 double bond}] - 759[\text{TAG w/ 7 double bonds}] \quad (1)$$

This model had a correlation of R^2_{adj} = 95.2%. The objective is to incorporate into this model the additional consideration of individual fatty acids (most specifically, *trans*-fats and unsaturated fatty acids more than 18 carbons in length), sample purity, and endogenous vitamin concentration. The selection of these factors and the methodology for their sequential inclusion in the model is discussed in chapter 5.

MATERIALS AND METHODS

Oxidation data

Acquisition of oxidation data used for this chapter is described in chapter 3 of this dissertation.

Standardized Summation of Area under the Curve

"Standardization Summation of Area under the Curve" (St. Sum AUC) term was calculated as described in chapter 3 according to the following equation (2):

$$\text{St. Sum AUC} = [PV_{AUC} + (6.31)CDT_{AUC} + (2.60)TBARS_{AUC} + (2.87)p\text{-}AnV_{AUC}]/4 \quad (2)$$

Regioisomeric Distribution of Fatty Acids in Triacylglycerols

Regioisomeric distribution of fatty acids in TAG was determined by hybrid mass spectrometry according to the methodology described by Nagy et al. (2012). For each sample, this data was used to calculate the concentrations of positional isomers, monoacylglycerols, free fatty acids, and TAG double bond distribution.

Quantification of trans-Fatty Acids and Unsaturated Fatty Acids Greater than 18 Carbons in Length

Fatty acids were quantified by gas chromatography, in accordance with the GC-FID-FAME procedure outlined in Badings and De Jong (1988).

Sample Purity

Sample purity was ascertained according to the specification sheets of the oil suppliers. Here, purity is meant simply to describe "single source" (e.g. canola oil, soybean oil), as in contrast to oil blends (e.g. confectionary blends, palm/coconut blends). It is not a quantitative term, and is treated as a binary variable.

Endogenous Vitamin Concentrations

Endogenous vitamin concentrations of the samples were evaluated by HPLC-MS, according to the method described in Nagy et al. (2007). The vitamins assessed were β-carotene, vitamin K, retinyl acetate, α-tocopherol, α-tocotrienol, β–tocopherol, γ-tocopherol, β-tocotrienol, γ-tocotrienol, σ-tocopherol, and σ–tocotrienol.

Model Building by Multiple Linear Regression Techniques

The method for factor selection and model-building was performed as discussed in chapter 5. Due to the binary nature of the sample purity consideration, the model was improved at that point by separating the single model into two models (one for pure samples and one for blends). The method for model improvement according to remaining factors was subsequently performed in tandem upon both the pure sample model and the model for the blends.

Determination of Specific Relative Effects of Variables

The coefficients in predictive models can provide meaningful information regarding the relative effects of variables. However, this information is often not effectively conveyed by a single choice of a good model. For example, certain variables may be eliminated from models

for the sake of simplicity, which results in the loss of information regarding the effect that variable may exhibit upon the outcome.

Therefore, this study produces multiple models following the addition of each new considered factor, for the single purpose of quantifying the effects indicated by coefficients. The method for acquiring these coefficients depended somewhat on the stage of model-building. Specifically, the techniques implemented were as follows:

Fatty Acid Data: Two summation terms were calculated for each sample from the fatty acid composition data. One describes the sum concentration of all TFA within the sample, and the other describes the sum concentration of all unsaturated fatty acids greater than 18 carbons in length (USFA>18C). Each of these two summed terms was then separately inputted directly as factors into the previous best model. The signs of the coefficients of these factors were recorded as indicators of each factor's observed correlatory effect upon oxidative stability.

Sample Purity: Purity was constructed as a binary variable and inputted directly into the previous best model. The magnitude of the coefficient here is without meaningful point of comparison, but the inverse of the sign is an indicator of the effect of purity upon stability.

Endogenous Vitamin Concentrations: The statistically significant variables of this factor were inputted directly into each of the previous best models (the pure sample model and the model for the blends). The relative magnitudes of the coefficients here are not meaningful due to different scales of magnitudes of occurrences for different vitamins. However, the inverse of the signs are

an indicator of the effect of the respective vitamins upon stability used in the model and are reported.

RESULTS AND DISCUSSION

Quantification of trans-Fatty Acids and Unsaturated Fatty Acids Greater than 18 Carbons in Length

Table 7.1 shows the concentrations of TFA for each of the 50 samples, and T**able 7.2** shows the concentrations of USFA>18C. These tables show the samples in order of ascending unsaturation. In neither case is there a clear pattern either in regards to sample unsaturation or oil type. TFA occur in dramatically higher quantities within the hydrogenated palm blend sample than within other samples, which is the expected result of partial hydrogenation. The lack of TFA in the hydrogenated oil blend sample suggests complete hydrogenation.

Sample Purity

Table 7.3 shows the categorization of samples according to pure or blended sample. There were 23 blends and 27 pure samples. The ranges and distributions of unsaturation are similar in both sample subsets.

Endogenous Vitamin Concentrations

Endogenous vitamin concentrations for β-carotene, vitamin K, retinyl acetate, α-tocopherol, α-tocotrienol, β–tocopherol, γ-tocopherol, β-tocotrienol, γ-tocotrienol, σ-tocopherol, and σ–tocotrienol are shown in **Table 7.4**. These concentrations generally do not show clear consistent patterns in regards to either sample unsaturation or oil type, with the exception of α-

tocopherol, which generally has higher concentrations in samples of higher unsaturation (R^2 = 44%).

Model Building by Multiple Linear Regression Techniques

The incorporation of trans-fatty acids and unsaturated fatty acids longer than 18 carbons into modeling efforts allowed for models of improved correlatory strength compared to the previous best model. The best chosen model including these considerations is as follows (3):

$$\text{St. Sum AUC} = 70 + 46.8[\text{MUFA}] + 180[\text{DiUFA}] + 556[\text{TriUFA}] - 18.5[\text{TAG w/ 1 double bond}] - 297[\text{TAG w/ 7 double bonds}] + 34303[\text{C22:1n9}] - 4760[\text{C18:3-trans}] \quad (3)$$

The correlation strength of the new model (R^2_{adj} = 96.1%) is a marginal improvement over the previous best model. The positive correlatory effect with oxidation of the long (>18C) fatty acid and the negative correlatory effect with oxidation of the *trans*-fatty acid are both consistent with the observed effect of their respective categorical assignments, which will be discussed in the next section.

The additional considerations of purity and vitamin concentrations allowed for the derivation of the following two models:

$$\text{St. Sum AUC (Blended Samples)} = 232 + 49.6[\text{MUFA}] + 146[\text{DiUFA}] + 1050[\text{TriUFA}] - 14.7[\text{TAG w/ 1 double bond}] - 259[\text{TAG w/ 7 double bonds}] - 1753[\text{C18:3-trans}] + 4146[\text{C22:1n9}] + 28.9[\gamma\text{-tocotrienol}] - 60.7[\alpha\text{-tocotrienol}] \quad (4)$$

$$\text{St. Sum AUC (Pure Samples)} = -415 + 48.3[\text{MUFA}] + 180[\text{DiUFA}] + 922[\text{TriUFA}] - 10.9[\text{TAG w/ 1 double bond}] - 834[\text{TAG w/ 7 double bonds}] - 1342[\text{C18:3-trans}] + 5063[\text{C22:1n9}] + 13.0[\gamma\text{-tocotrienol}] - 22.2[\alpha\text{-tocotrienol}] \quad (5)$$

These models are shown graphically in **Figures 7.1** and **7.2**, respectively. The improvements to predictive strength by the incorporation of sample purity and vitamin concentrations are fairly minimal (R^2_{adj} = 97.1% for the model of oil blends and R^2_{adj} = 96.2% for the model of pure samples), but still provide some elucidation regarding the effect and importance of these factors. Notably, the patterns of signs and magnitudes for the possible variables are consistent between both of these models, and also with those of the previous models demonstrated throughout this dissertation. This noted consistency serves somewhat as a validation step of the true correlatory effects of these variables.

These two models also suggest a negative correlatory effect of γ-tocotrienol with observed stability and a positive correlatory effect of α-tocotrienol with observed stability – observations consistent with the direct determination of variable effects discussed in the following section. Concentrations of β-carotene, vitamin K, retinyl acetate, α-tocopherol, β–tocopherol, γ-tocopherol, β-tocotrienol, σ-tocopherol, and σ–tocotrienol all failed to prove statistically significant or yield benefit to the models.

The final two models, as well as the ones preceding, present predictive strength greater than that typically expected for predictions of oxidative stability. This is likely due to both the comprehensiveness of oxidative data collected, and the comprehensive nature of the St. Sum AUC – the effect being a reduced likelihood of overemphasis on aberrant behavior. It is believed the resulting models could be of immediate practical use for the comparison of expected stability of edible fats and oils. The best choice of model among those presented will depend on

practical considerations (budget for data collection, etc.), preferences for model simplicity, and the degree of predictive strength required.

Determination of Specific Relative Effects of Variables

Table 7.5 shows the observed effect (as determined by sign of coefficient) of the considered variables following their direct inputs into the previous best models. TFA, sample purity, and α-tocotrienol were all associated with improved stability. USFA>18C and γ-tocotrienol were associated with impaired stability. The effects attributed to these variables are consistent with those in all final reported models. This consistency provides evidence of the validity of these observed effects.

The improvements to stability associated with *trans*-fatty acids are well-documented (Kamal-Eldin, 2006), so the results here are in accordance with expectations. The observation of impaired stability associated with the concentration of USFA>18C is a more novel finding. Only one similar examination could be found in scientific literature, which noted a similar effect (Kamal-Eldin, 2006). In both cases, it is important to remember that the observed effects are meant to be interpreted as autonomous of their contributions to sample unsaturation - meaning that the length (rather than simply its contribution of additional double bonds to the sample) of the unsaturated fatty acid is associated with oxidative stability. This observed association is in line with previous observations of the likely importance of steric hindrances and physical proximities to autoxidation (as discussed in previous chapters of this dissertation). It is believed this factor deserves further scientific investigation.

The observation of a relatively higher expectation of stability in pure oil samples is without direct comparison in scientific literature. However, the observed effect is not unexpected,

considering the previously discussed tendency of unadulterated single-source foods to generally exhibit nearly optimized stability (Kamal-Eldin, 2006; Mariod et al., 2005; Ramadan and Wahdan, 2012). The implication of such a finding is that minor constituents of the oil (that were either unobserved in this study or simply not included in the models) are in somewhat more favorable concentrations in the case of the single-source oils. This is reasonable given the tendency of plants to evolve towards a minimization of oxidative stress (Scartezzini and Speroni, 2000).

Neither the positive association with stability of α-tocotrienol nor the negative stability association with stabilityof γ-tocotrienol can be considered unexpected, as both such effects are frequently attributed to of these compounds as well as their related species (Carocho and Ferreira, 2013). However, opposite effects of these species of antioxidants are observed in literature as well, which indicates the inconsistencies of the reported effects of natural antioxidants (Carocho and Ferreira, 2013). The effect observed here is most likely less about the inherent effects of these compounds within edible oil, and more about their respective endogenous concentrations in the oils of this sample set relative to optimal levels. Samples which had higher levels of α-tocotrienol marginally outperformed the expectations of stability according to the previous models, which suggests the other samples may have generally contained this compound in concentrations below optimal levels. For γ-tocotrienol, the inverse is true. This observation may merit further investigation in the form of controlled additions of these compounds to this set of edible oil samples.

CONCLUSIONS

Concentrations of USFA>18C, TFA, α-tocotrienols, and γ-tocotrienols were all significant predictors of oxidative stability. Models were also improved by distinguishing pure samples from blends. Final models were highly predictive of oxidative stability (R^2_{adj} = 97.1% for the model of oil blends and R^2_{adj} = 96.2% for the model of pure samples). Independent of sample unsaturation, TFA, sample purity, and α-tocotrienol were all associated with improved stability. USFA>18C and γ-tocotrienol were associated with impaired stability. The models presented here are believed to provide a practical tool for the prediction of oxidative stability of edible fats and oils. Moreover, the observed effects of these variables provide further evidence of the importance of proximity and physical hindrance upon oxidative reactions. These factors may merit further investigation in the form of controlled experiments and in the development of oils optimized for stability.

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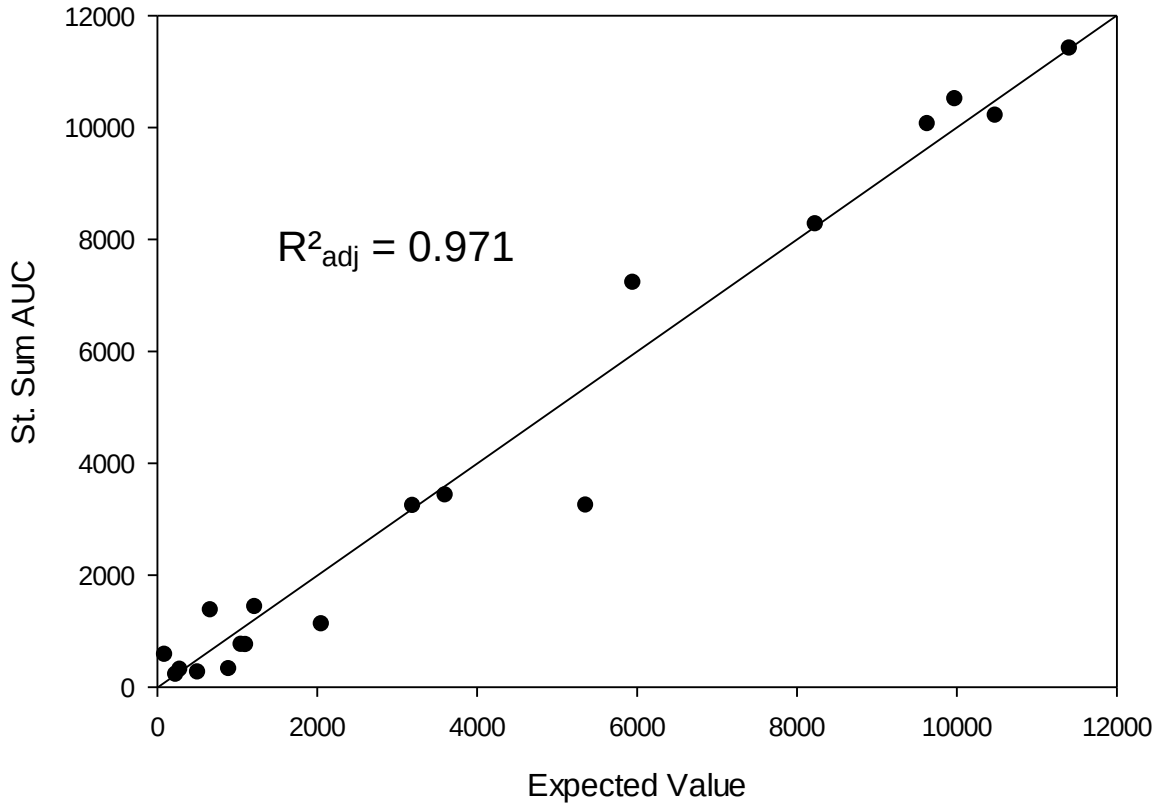

Figure 7.1 Model for the prediction of oxidative stability within blended fat and oil samples. This is the correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA, triacylglycerol double bond distribution, fatty acid composition, and endogenous vitamin concentrations.

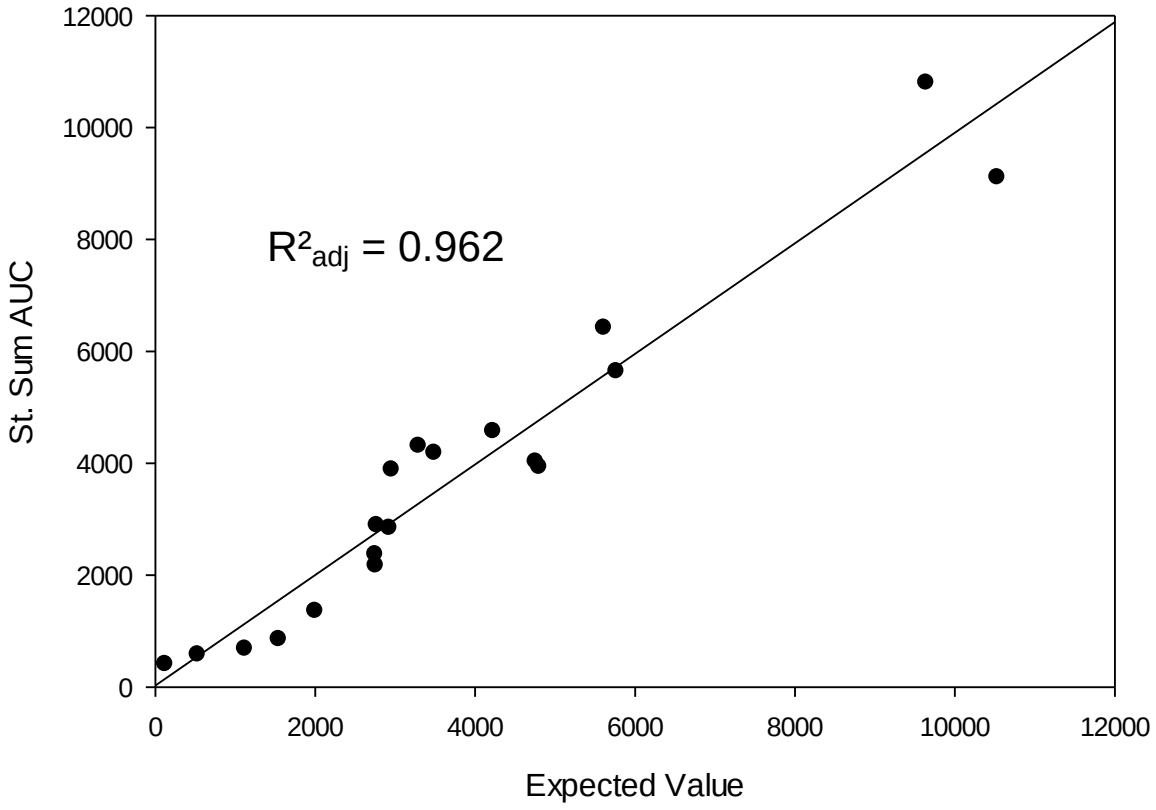

Figure 7.2 Model for the prediction of oxidative stability within pure (i.e. single-source) fat and oil samples. This is the correlation between St. Sum AUC (term for oxidative stability) and the expected value of a predictive model according to concentrations of MUFA, DiUFA, and TriUFA, triacylglycerol double bond distribution, fatty acid composition, and endogenous vitamin concentrations.

Table 7.1 *trans*-Fatty acids in samples (g/100 g sample)

Sample	C18:1(*trans*)	C18:2(*trans*)	C18:3(*trans*)	Total *trans*-fat
MCTs 1	0.00	0.00	0.00	0.00
MCTs 2	0.06	0.00	0.00	0.06
Coconut Oil	0.06	0.02	0.00	0.09
Cocoa Butter Substitute	0.17	0.00	0.00	0.17
Hydrogenated Oil Blend	0.00	0.00	0.00	0.00
Palm Kernel Stearin Oil	5.93	0.33	0.00	6.25
Hydrogenated Palm Blend	49.15	1.44	0.00	50.59
Coconut Oil	0.07	0.05	0.00	0.12
Palm Kernel Oil	0.08	0.29	0.00	0.37
Palm Kernel Olein Oil	0.15	0.23	0.00	0.38
Palm Stearin Oil	0.03	0.09	0.00	0.12
Cocoa Butter Substitute	0.06	0.18	0.00	0.24
Cocoa Butter Equivalent 1	0.09	0.06	0.00	0.14
Palm/Coconut Mix	0.11	0.15	0.00	0.26
Cocoa Butter Equivalent 2	0.04	0.34	0.00	0.38
Cocoa Butter Equivalent 3	0.00	0.07	0.00	0.07
Shea Oil/Palm	0.07	0.54	0.09	0.70
Palm Oil 1	0.04	0.15	0.18	0.37
Palm Oil 2	0.04	0.18	0.22	0.43
Palm Oil 3	0.02	0.12	0.34	0.49
Palm/Coconut High Oleic	0.08	0.20	0.00	0.29
Palm Olein Oil 1	0.03	0.21	0.27	0.51
Cocoa Butter Replacer	0.00	0.14	0.31	0.46
Palm Oil 4	0.00	0.04	0.00	0.04
Palm Olein Oil 2	0.00	0.00	0.00	0.00
Palm Olein Oil 3	0.08	0.07	0.00	0.15
Palm Olein Oil 4	0.02	0.02	0.00	0.04
Palm/Soybean/Canola	0.03	0.12	0.00	0.15
Palm Olein Oil 5	0.06	0.20	0.03	0.29
Palm Olein Oil 6	0.09	0.57	0.00	0.66
Oil Mix A	0.00	0.34	0.00	0.34
Oil Mix B	0.05	0.55	0.05	0.64
Olive Oil 1	0.04	0.47	0.05	0.56
Olive Oil 2	0.04	0.20	0.00	0.24
Sunflower Oil High Oleic 1	0.06	0.11	0.07	0.25
Sunflower Oil High Oleic 2	0.00	0.08	0.00	0.08
Oil Mix C	0.00	0.29	0.04	0.32
Canola Oil 1	0.00	0.30	0.00	0.30
Corn/Canola Mix	0.07	0.39	0.04	0.51
Corn/Canola Mix D	0.03	0.14	0.06	0.23
Canola/Sunflower/Corn	0.04	0.06	0.00	0.10
Canola Oil 2	0.00	0.33	0.00	0.33
Canola Oil 3	0.04	0.28	0.49	0.80
Corn Oil 1	0.00	0.29	0.09	0.38
Corn/Canola Mix E	0.00	0.11	0.52	0.63
Corn Oil 2	0.02	0.08	0.42	0.53
Corn Oil 3	0.02	0.13	0.35	0.50
Corn Oil 4	0.00	0.14	0.06	0.21
Sunflower Oil	0.00	0.23	0.00	0.23
Soybean Oil	0.00	0.10	0.06	0.16

Table 7.2 Unsaturated fatty acids longer than 18 carbons in samples (g/100 g sample)

Sample	C22:1n-9	C22:5n-3	C24:1n-9	Total Unsaturated Fatty Acids > 18 C
MCTs 1	0.00	0.00	0.00	0.00
MCTs 2	0.00	0.00	0.00	0.00
Coconut Oil	0.00	0.00	0.00	0.05
Cocoa Butter Substitute	0.00	0.00	0.00	0.00
Hydrogenated Oil Blend	0.00	0.00	0.00	0.00
Palm Kernel Stearin Oil	0.00	0.00	0.00	0.06
Hydrogenated Palm Blend	0.00	0.00	0.00	0.00
Coconut Oil	0.00	0.00	0.00	0.04
Palm Kernel Oil	0.00	0.00	0.00	0.08
Palm Kernel Olein Oil	0.00	0.00	0.00	0.08
Palm Stearin Oil	0.00	0.00	0.00	0.04
Cocoa Butter Substitute	0.00	0.00	0.00	0.11
Cocoa Butter Equivalent 1	0.00	0.00	0.00	0.05
Palm/Coconut Mix	0.00	0.00	0.00	0.09
Cocoa Butter Equivalent 2	0.00	0.00	0.03	0.20
Cocoa Butter Equivalent 3	0.00	0.00	0.00	0.35
Shea Oil/Palm	0.00	0.00	0.00	0.15
Palm Oil 1	0.07	0.00	0.00	0.44
Palm Oil 2	0.00	0.00	0.05	0.59
Palm Oil 3	0.11	0.00	0.00	0.74
Palm/Coconut High Oleic	0.00	0.00	0.00	1.07
Palm Olein Oil 1	0.07	0.00	0.05	0.68
Cocoa Butter Replacer	0.04	0.00	0.08	0.89
Palm Oil 4	0.00	0.00	0.00	0.04
Palm Olein Oil 2	0.00	0.00	0.00	0.00
Palm Olein Oil 3	0.00	0.00	0.00	0.04
Palm Olein Oil 4	0.00	0.00	0.00	0.09
Palm/Soybean/Canola	0.00	0.00	0.00	0.12
Palm Olein Oil 5	0.00	0.00	0.00	0.08
Palm Olein Oil 6	0.00	0.00	0.00	0.12
Oil Mix A	0.00	0.00	0.00	0.13
Oil Mix B	0.00	0.00	0.00	0.12
Olive Oil 1	0.00	0.00	0.00	0.14
Olive Oil 2	0.00	0.00	0.00	0.15
Sunflower Oil High Oleic 1	0.00	0.00	0.00	0.24
Sunflower Oil High Oleic 2	0.00	0.00	0.00	0.22
Oil Mix C	0.00	0.00	0.00	0.17
Canola Oil 1	0.00	0.00	0.00	0.14
Corn/Canola Mix	0.00	0.00	0.00	0.15
Corn/Canola Mix D	0.00	0.00	0.00	0.28
Canola/Sunflower/Corn	0.00	0.03	0.00	0.25
Canola Oil 2	0.00	0.04	0.00	0.33
Canola Oil 3	0.00	0.00	0.00	0.20
Corn Oil 1	0.00	0.00	0.00	0.28
Corn/Canola Mix E	0.10	0.00	0.13	1.39
Corn Oil 2	0.08	0.00	0.00	1.20
Corn Oil 3	0.11	0.00	0.00	0.75
Corn Oil 4	0.00	0.00	0.00	0.27
Sunflower Oil	0.00	0.00	0.00	0.14
Soybean Oil	0.00	0.00	0.00	0.25

Table 7.3 Sample set according to classification as pure or blended

Blended Samples		Pure Samples	
Sample Name	**CIV**	**Sample Name**	**CIV**
MCTs 1	0	Coconut Oil	0.1
MCTs 2	0	Palm Kernel Stearin Oil	6.25
Cocoa Butter Substitute	0.15	Coconut Oil	8.45
Hydrogenated Oil Blend	1.33	Palm Kernel Oil	16.2
Hydrogenated Palm Blend	6.71	Palm Kernel Olein Oil	22.5
Cocoa Butter Substitute	31.5	Palm Stearin Oil	30.7
Cocoa Butter Equivalent 1	31.6	Palm Oil 3	47.8
Palm/Coconut Mix	31.6	Palm Olein Oil 1	48.1
Cocoa Butter Equivalent 2	31.9	Palm Oil 4	50
Cocoa Butter Equivalent 3	32.1	Palm Olein Oil 2	52
Shea Oil/Palm Blend	40.2	Palm Olein Oil 3	53.7
Palm Oil 1	42.1	Palm Olein Oil 4	54.5
Palm Oil 2	42.1	Palm Olein Oil 5	57.2
Palm/Coconut High Oleic	47.9	Palm Olein Oil 6	58.6
Cocoa Butter Replacer	49	Olive Oil 1	74.9
Palm/Soybean/Canola	57.1	Olive Oil 2	76.6
Oil Mix A	62.5	Sunflower Oil High Oleic 1	81.7
Oil Mix B	64.6	Sunflower Oil High Oleic 2	86
Oil Mix C	94.9	Canola Oil 1	95.4
Corn/Canola Mix	96.3	Canola Oil 2	103
Corn/Canola Mix D	96.4	Canola Oil 3	104
Canola/Sunflower/Corn	97.6	Corn Oil 1	107
Corn/Canola Mix E	109	Corn Oil 2	110
		Corn Oil 3	111
		Corn Oil 4	118
		Sunflower Oil	120
		Soybean Oil	120

Table 7.4 Endogenous vitamin concentrations in samples (nmoles/L oil)

Sample	β-Carotene	Vitamin K	Retinyl acetate	Tocopherols				Tocotrienols			
				α	β	γ	σ	α	β	γ	σ
MCTs 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MCTs 2	0.0	0.0	0.0	1.9	0.0	1.9	0.0	0.0	0.0	0.0	0.0
Coconut Oil	0.0	0.0	0.0	5.1	0.0	4.0	1.3	0.0	0.0	0.0	0.0
Cocoa Butter Substitute	0.0	0.0	0.0	18.6	1.8	8.8	2.9	1.3	0.0	2.4	0.0
Hydrogenated Oil Blend	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palm Kernel Stearin Oil	2.6	0.0	0.0	15.3	1.9	58.6	18.6	0.0	0.0	0.0	3.1
Hydrogenated Palm Blend	0.0	0.0	0.0	5.6	1.6	32.4	11.0	0.0	0.0	0.0	0.0
Coconut Oil	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	5.3	2.7
Palm Kernel Oil	3.4	0.0	0.0	58.7	0.0	7.8	2.9	51.3	10.3	95.6	17.0
Palm Kernel Olein Oil	7.1	0.0	0.0	95.9	0.0	7.6	0.0	68.0	12.2	137.4	27.8
Palm Stearin Oil	0.0	0.0	0.0	6.0	0.0	0.0	0.0	5.9	0.0	17.3	5.1
Cocoa Butter Substitute	3.5	0.0	0.0	63.7	0.0	0.0	0.0	56.0	11.1	124.4	23.7
Cocoa Butter Equivalent 1	0.0	0.0	0.0	4.7	0.0	0.0	0.0	0.0	0.0	4.5	2.2
Palm/Coconut Mix	5.0	0.0	0.0	99.9	0.0	5.4	0.0	94.4	16.0	171.2	32.2
Cocoa Butter Equivalent 2	6.8	0.0	0.0	97.1	0.0	2.6	0.0	86.8	0.0	145.5	36.5
Cocoa Butter Equivalent 3	5.8	0.0	0.0	136.7	3.9	256.4	32.7	78.4	15.4	150.4	35.5
Shea Oil/Palm	5.2	0.0	0.0	123.9	7.6	388.5	147.2	79.9	14.6	154.5	28.7
Palm Oil 1	4.2	0.0	0.0	232.5	4.6	102.4	2.1	67.2	12.3	106.4	20.6
Palm Oil 2	2.6	1.1	0.0	235.2	5.8	648.3	21.3	32.5	9.4	73.1	15.9
Palm Oil 3	0.0	1.1	0.0	418.2	14.8	212.3	4.1	0.0	0.0	0.0	0.0
Palm/Coconut High Oleic	0.0	1.4	0.0	273.2	6.1	345.3	8.8	2.6	0.0	3.1	0.0
Palm Olein Oil 1	2.1	0.0	0.0	190.8	4.1	565.2	17.5	31.6	0.0	60.3	15.3
Cocoa Butter Replacer	0.0	1.3	0.0	190.7	7.3	912.2	53.7	5.2	0.0	14.5	1.1
Palm Oil 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palm Olein Oil 2	0.0	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Palm Olein Oil 3	0.0	0.0	0.0	4.1	0.0	0.0	0.0	26.8	7.3	12.1	2.5
Palm Olein Oil 4	0.0	0.0	0.0	2.4	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Palm/Soybean/Canola	0.0	0.0	2.0	3.6	0.0	0.0	0.0	16.3	0.0	4.2	0.0
Palm Olein Oil 5	8.8	0.0	0.0	23.6	1.5	2.7	0.0	19.0	8.4	83.0	17.7
Palm Olein Oil 6	3.3	0.0	0.0	80.0	0.0	1.8	0.0	83.4	0.0	114.7	25.6
Oil Mix A	7.9	0.0	0.0	105.1	0.0	0.0	0.0	129.4	22.7	245.9	50.5
Oil Mix B	5.4	0.0	0.0	108.7	1.4	0.0	0.0	86.0	14.6	179.1	29.2
Olive Oil 1	6.9	0.0	0.0	100.4	0.0	0.0	0.0	104.9	16.7	199.4	33.6
Olive Oil 2	17.1	0.0	0.0	133.0	0.0	0.0	0.0	162.9	23.9	337.9	50.4
Sunflower Oil High Oleic 1	0.0	0.0	0.0	164.6	1.9	13.0	0.0	0.0	0.0	0.0	0.0
Sunflower Oil High Oleic 2	0.0	0.0	0.0	656.2	17.9	2.8	0.0	0.0	0.0	0.0	0.0
Oil Mix C	10.9	0.0	0.0	199.1	3.2	70.6	27.1	150.4	24.6	319.2	59.1
Canola Oil 1	7.3	0.0	0.0	144.2	0.0	0.0	0.0	136.2	20.8	276.2	47.4
Corn/Canola Mix	7.3	0.0	0.0	139.6	0.0	1.9	0.0	141.4	20.6	234.1	50.5
Corn/Canola Mix D	0.0	0.0	0.0	109.3	0.0	6.1	0.0	0.0	0.0	0.0	0.0
Canola/Sunflower/Corn	0.0	0.0	0.0	468.2	10.4	5.2	1.3	0.0	0.0	0.0	0.0
Canola Oil 2	0.0	0.0	0.0	294.9	7.4	836.7	19.5	11.9	0.0	19.2	1.8
Canola Oil 3	0.0	2.1	0.0	201.1	29.4	767.5	329.6	0.0	0.0	3.0	1.3
Corn Oil 1	0.0	0.0	0.0	416.0	15.3	768.7	22.2	10.2	0.0	10.8	0.0
Corn/Canola Mix E	0.0	1.7	0.0	282.5	3.5	419.1	7.0	0.0	0.0	0.0	0.0
Corn Oil 2	0.0	1.6	0.0	262.3	3.4	391.0	8.1	0.0	0.0	2.3	0.0
Corn Oil 3	0.0	1.0	0.0	380.3	12.0	174.6	3.1	0.0	0.0	0.0	0.0
Corn Oil 4	0.0	0.0	0.0	300.4	14.0	10.6	1141.5	0.0	23.0	36.4	1.5
Sunflower Oil	0.0	0.0	0.0	560.1	0.0	18.7	11.4	0.0	0.0	4.4	0.0
Soybean Oil	0.0	0.0	0.0	362.0	34.9	14.2	1208.7	0.0	58.0	45.2	2.7

Table 7.5 Observed correlatory effects upon oxidative stability within samples

Variable	Observed Correlatory Effect
trans-Fatty acids	Improved stability
Unsaturated fatty acids longer than 18 carbons	Impaired stability
Purity (not a blend)	Improved stability
α-Tocotrienol	Improved stability
γ-Tocotrienol	Impaired stability

CHAPTER 8

CONCLUSIONS

Lipid oxidation has been a longstanding concern for the food industry. Demonstrated efforts in scientific literature to predict, model, and enhance oxidative stability have suffered from varying and inconsistent results. Among the possible explanations of this phenomenon are incomprehensive assessments of oxidation and inconsistent techniques by which to define oxidative stability. Another complicating issue is the possible synergies and antagonisms that may occur between the factors which affect stability – thereby possibly rendering the isolated effects of individual factors incomparable across differing studies. Redundancies within factors (particularly those which signify sample unsaturation) also cause difficulties in determining specificity in factor effects. This study represents an attempt to address each of these concerns and to produce models that are not only predictive of oxidative stability, but also effectively elucidate the effects of factors that have not been previously well evidenced.

The comprehensive assessment of lipid oxidation products by multiple methods throughout extended storage allowed for a good comparison of numerical interpretations of curves. It was determined that AUC outperformed six other quantitative interpretations (maximum value, time of maximum value, time of 20% maximum value, integrated area prior to 20% maximum value, maximum value/time of maximum value, and slope of tangent line during growth phase) in regards to vulnerability to instability among assays used to assess oxidation. From this, a combined quantitative term, the St. Sum AUC, was derived – representing a comprehensive quantitative definition of oxidative stability. No similar comprehensive term is found in prior

literature, and the term could present a useful technique for scientists to better assess oxidative stability in a way that is comparable across studies. For the sake of practicality, a good approximation of this term was found to be possible according to the acquisition of only three data points throughout accelerated storage.

Regression models demonstrated that this term of oxidative stability could be predicted quite well according to the calculated iodine value of a sample (R^2 = 87.3%), and that this predictive strength was improved (R^2_{adj} = 91.5%) by the individual consideration of the concentrations of MUFA, DiUFA, and TriUFA. The model indicated the relative effect upon magnitude of oxidation of MUFA:DiUFA:TriUFA to be approximately 1:3:12 – substantially greater than that of their relative degrees of unsaturation. The results suggest that the combined presence of multiple double bonds on individual fatty acids is associated with impaired oxidative stability.

Efforts to model the additional effects of multiple composition factors presented significant statistical challenges due to very high redundancies between many factors. A systematic approach of sequential reduction of model variance was ultimately implemented with the purpose of elucidating the possible unique effects of the factors. The results of this approach yielded models of good consistency (in regards to the observed effects of the included variables), and very high correlatory strengths. In order, the models were improved by the sequential consideration of the following factors: (1) fatty acid composition, (2) TAG double bond distribution, (3) sample purity, and (4) endogenous vitamin concentration. Positional isomer data, monoacylglycerol concentration, and free fatty acid concentration were also examined, but were not found to be significant factors in the investigated sample set.

The concentrations of TAG containing one, two, and seven double bonds were positively associated with stability, and the concentrations of TAG containing three, four, five, and six double bonds were negatively associated with stability. TFA, sample purity, and α-tocotrienol were all associated with improved stability, and USFA>18C and γ-tocotrienol were associated with impaired stability. The observed effects of TFA and sample purity met expectations, while the effects of USFA>18C and TAG double bond distribution represent possibly useful new findings. These findings reasonably fit (and perhaps expand upon) our current understanding of the importance of proximities and physical hindrances to lipid oxidation.

The intention of the sequential modeling was to allow for these effects of factors to be interpreted in isolation of their possible contributions to (or linear associations with) sample unsaturation. The consistency of these observed effects throughout the model-building process served as a validation for the truth of these effects, but the extreme redundancy in the factors does still present cause for reasonable uncertainty in the final interpretations. It is believed, however, that the models demonstrated in this document represent a useful and reliable means to predict oxidative stability within commercial edible oils and fats, and that food industry members could benefit by the consideration of their use. Moreover, it is believed that the isolated effects of the factors delineated here merit further investigation according to studies designed with controlled variables. Finally, it is hoped that the quantitative term for oxidative stability, the St. Sum AUC, is considered for use in further studies.

APPENDIX A

CALCULATION OF COEFFICIENTS FOR STANDARDIZED SUMMATION OF AUC

The purpose of the calculation of these coefficients was to produce a mathematical term that provides comparable quantitative weight to each of the four AUC values associated with the four oxidation assessment assays. Each of these four assays generates results in different units and upon different scales of magnitude. It was therefore necessary to create coefficients that account for these differences in magnitude scale and allow each of the four assays to have comparable representation within the final term.

In the examination of the 50 samples assessed in this study, the average AUC values for each assay are as follows:

Average $PV_{AUC} = 4376$

Average $CDT_{AUC} = 694$

Average $TBARS_{AUC} = 1683$

Average $p\text{-}AnV_{AUC} = 1525$

PV AUC values are of the highest magnitude and were therefore assigned a coefficient of 1.00. The PV AUC values are of an average magnitude 6.31 times greater than those of CDT, 2.60 times greater than those of TBARS, and 2.87 times greater than those of *p*-AnV. These multiples therefore became the coefficients for the AUC values of these respective assays in the St. Sum AUC term.