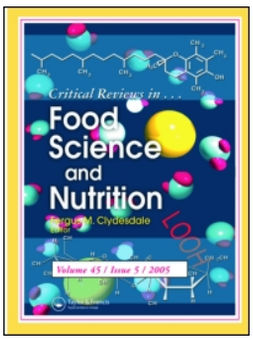

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Lipid Oxidation in Low-moisture Food: A Review

LEANN BARDEN and ERIC A. DECKER

Department of Food Science, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Overly high intake of saturated fat is an international problem contributing to global health issues. Low-moisture snacks account for a nutritionally significant proportion of the saturated fat in the diet, making these foods a key target for improving consumers' health. However, it is not currently feasible to maintain the same oxidative shelf life when replacing saturated fats with unsaturated fats, which are generally perceived to be more heart-healthy. This article summarizes current theories and available research on lipid oxidation in low-moisture foods in order to lay the groundwork for new lipid oxidation rate-reduction strategies. Research deficits needing attention and new methods for assessing lipid oxidation in low-moisture foods are also discussed.

Keywords Saturated fat, monolayer, lipid oxidation kinetics, antioxidants, surface oxidation, shelf life, snack foods

INTRODUCTION

Lipid oxidation is a problem in food because it produces compounds that degrade product quality, alter textural properties, and adversely affect the color and nutrition of a food product. In foods, unsaturated fatty acids—typically those once esterified to the glycerol backbone of a triacylglycerol (TAG) or phospholipid—decompose into volatile compounds with low molecular weights that produce off-aromas associated with rancidity. Many of these volatile lipid oxidation products are detectable by humans at the parts per million and even parts per billion threshold (Labuza and Dugan, 1971). Ultimately, lipid oxidation reduces shelf life and therefore causes food spoilage, an important factor in food security as understood to be the availability to, and accessibility of, high-quality food.

Susceptibility of fatty acids to lipid oxidation increases with the degree of unsaturation due to increasingly lower bond dissociation energies of methylene-interrupted carbons (McClements and Decker, 2008). Polyunsaturated fatty acids (PUFAs) are generally viewed as healthier for consumers, but if manufacturers abide by the latest Dietary Guidelines (WHO, 2003; EFSA, 2007; U.S. Dept. of Health and Human Services, 2010) to reduce solid fats by simply substituting PUFAs for saturated fats, they risk severely decreasing food acceptability and shelf life.

Although rancidity has been studied in many types of foods, little systematic research has been conducted on low-moisture foods—those with water activity (a_w) below 0.5 and whose shelf life is primarily limited by lipid oxidation and non-enzymatic browning (Labuza et al., 1970). Unfortunately, most research relating a_w and lipid oxidation rates dates back to the 1970s with little recent research. It is important to expand studies in this area since surveys have shown that grain-based desserts and snacks—including many low-moisture foods like cookies and granola bars—are among the top three contributors of saturated fat to the American diet (National Cancer Institute, 2010b). Crackers rank among the top 15 fat-contributing foods, specifically accounting for 1.5% of total solid fat consumption among youth aged 2–18 years (National Cancer Institute, 2010a). This suggests that if the nutritional profiles of low-moisture products could be improved by substituting their saturated fatty acids with unsaturated fatty acids, then this could have an important impact on consumer health. However, this is not currently feasible because many of the basic mechanisms of lipid oxidation in low-moisture foods are still not well understood. Thus, there is a clear need to revisit this topic and understand where, how, and why lipid oxidation occurs in low-moisture systems. Only then can manufacturers implement abatement and reduction strategies to improve consumer health.

Address correspondence to Eric A. Decker, Department of Food Science, University of Massachusetts Amherst, 228 Chenoweth Laboratory, 102 Holdsworth Way, Amherst, MA 01003, USA. E-mail: edecker@foodsci.umass.edu

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MECHANISM OF LIPID OXIDATION

The process by which lipid oxidation proceeds has been extensively reviewed elsewhere (Labuza and Dugan, 1971; Kamal-Eldin, 2003; Frankel, 2005; McClements and Decker, 2008). Briefly, lipid oxidation is characterized by three phases: initiation, propagation, and termination. Initiation begins when a hydrogen is abstracted from a fatty acid, thereby generating an alkyl radical (R•). Typically, abstraction occurs at a methylene-interrupted carbon of a PUFA, where the covalent bond strength between hydrogen and its methylene carbon is reduced. As more double bonds are added to the fatty acids, oxidation susceptibility increases due to the addition of more methylene-interrupted carbon reaction sites. Research has shown that the addition of one double bond to a PUFA at least doubles the rate at which the fatty acid oxidizes (Holman and Elmer, 1947; Bolland, 1948; Buttery et al., 1961), with the *cis* form often oxidizing more readily than the *trans* form (Sargis and Subbaiah, 2003). Therefore, the likelihood of lipid oxidation and formation of deleterious sensory products increases with increasing unsaturation. Enzymes, metals, metalloproteins, light, high processing temperatures, and irradiation can all promote lipid oxidation in the initiation stage as they primarily accelerate oxidation via reactions that produce free radicals and/or reactive oxygen species.

After initial hydrogen abstraction from the fatty acid, the energy of the resulting alkyl radical is decreased by isomerization to form conjugated double bonds. This is followed by propagation where the alkyl radical undergoes addition with atmospheric oxygen to form a peroxyl radical (ROO•). This peroxyl radical has sufficient energy to promote the abstraction of hydrogen from another unsaturated fatty acid, thus forming another alkyl radical and a lipid hydroperoxide (ROOH). Thus, propagation involves the transfer of a free radical from one fatty acid to another. Lipid hydroperoxides themselves, however, are not volatile and therefore do not contribute to rancid aromas. β-scission reactions—promoted by heat, light, and transition metals—cause decomposition of the lipid hydroperoxides into alkoxyl radicals (RO•). This step represents the formation of a second free radical that can attack additional fatty acids and causes an exponential increase in oxidation rates. In addition, alkoxyl radicals are high energy allowing them to break the aliphatic chain of the fatty acid and generate the low molecular weight volatiles that are associated with the characteristic rancid smell of oxidized fats. Many researchers measure both hydroperoxide content and aldehyde content in final products when determining the extent of lipid oxidation to monitor both propagation and β-scission reactions.

During the termination phase, two radicals react to form one, non-radical molecule, such as fatty acid dimers, trimers, and oligomers. Consequently, these polymers often either precipitate or lead to increases in oil viscosity. Polymeric products are common in frying oils, where the high temperatures reduce oxygen solubility and thus minimize hydroperoxide formation and therefore β-scission reactions. Termination reactions are not as important in other foods because higher oxygen allows β-scission reactions to predominate and because most foods are deemed rancid before termination reactions are significant.

When monitoring lipid oxidation over time, one typically observes a lag phase where accumulation of lipid oxidation products is slow. This lag phase is the result of the slow formation of free radicals prior to hydroperoxide accumulation and β-scission reactions and also the presence of antioxidants that are preferentially oxidized and thus prevent free radicals from attacking fatty acids. Food manufacturers strive to maximize the duration of the lag phase where the concentration of the products responsible for rancidity are below sensory threshold levels. Therefore, the lag phase is the best representation of the oxidative shelf life of a food product.

LIPID OXIDATION KINETICS

Kinetics of lipid oxidation are often analyzed when determining the shelf life of a product. Rate constants must be determined experimentally and depend upon parameters, such as temperature, pH, oxygen concentrations, surface area, and ionic strength. Oxidative reactions are fastest—and thus, shelf life experiments are shortest—at high temperatures, but using high temperatures runs the risk of changing factors, such as a_w, oxygen solubility, and partial pressure, and/or forming antioxidative side reaction products like those from Maillard browning or caramelization. Thus, it is strongly recommended that kinetic studies be conducted at multiple temperatures (Ragnarsson et al., 1977; Sullivan et al., 2011). As expected, rate constants determined under cycling temperatures differ from those determined under constant temperatures (Labuza and Bergquist, 1983).

Lipid oxidation reaction kinetics are not simple since each step—initiation, propagation, and termination—has its own rate constant (Labuza and Dugan, 1971). Even when considering the kinetics of hydroperoxide formation in commercial fish oil products, Sullivan et al. (2011) found the data fit first-order kinetics only within certain temperature ranges and depending on the particular PUFA composition of the oil. To simplify the complications of lipid oxidation kinetics, some scientists assume a linear approach when, in fact, the overall lipid oxidation reaction does not follow simple first-order kinetics. For example, after the lag phase, the reaction is exponential. Foods with the same duration of lag phase—like samples A and B in Fig. 1—may exhibit different rates of oxidation in the exponential phase. After the lag phase, sample B initially oxidizes at a slower rate than sample A but then continues to oxidize during storage. Why two foods would oxidize by such different kinetics could be due to many reasons. For example, product A could have very low levels of PUFAs. Therefore, after oxidation started it could rapidly end as substrate is consumed and thus no more lipid oxidation products

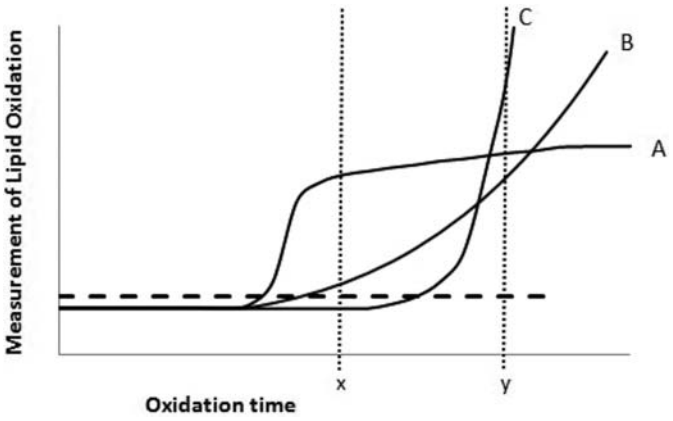

Figure 1 Example of oxidation curves for three different compounds where the *y*-axis might represent a measurement of primary or secondary oxidation products. At values above the sensory cut-off line, trained panelists found the product unacceptably oxidized.

would be formed. In sample B, there could be large amounts of PUFAs and thus oxidation products would continue to increase during prolonged storage. Finally, sample C depicts a sample that is stable for a long period (longer lag phase) but then rapidly oxidizes. For instance, perhaps the sample was manufactured with tocopherols. These antioxidants would extend the lag phase but once they were consumed by oxidation, fatty acid oxidation products would be produced rapidly.

Some researchers misinterpret shelf life estimations in lipid oxidation kinetics because they do not evaluate their data with a sensory perspective in mind. The shelf life of a product that is susceptible to lipid oxidation is determined when a consumer can detect lipid oxidation volatiles that impact flavor. Since some lipid oxidation products have very low sensory threshold values, sensory perception of rancidity can sometimes occur prior to being able to chemically detect oxidation products. However, once lipid oxidation products are detected (the end of the lag phase), then it is highly likely that the sensory properties of the product are compromised. From Fig. 1, we can only gather that compound C has the longest lag time and the best shelf life. Compounds A and B have the same lag time but different rates of oxidation following the lag period. The mistake made in numerous publications is that samples are compared after the lag phase. If this is done at time X, then one would conclude that the shelf life was in the order of C>B>A. However, if comparisons are made at time Y, the conclusions would be B>A>C. However, a comparison at time Y does not determine shelf life since all the products are rancid. Therefore, the length of the lag period is the most important factor in determining shelf life. If fortunate enough to have sensory data corresponding to the chemical data, then it matters only whether the extent of oxidation is above or below the sensory cut-off line.

Another common approach to simplify the complexity of oxidation kinetics is to take data like that illustrated in Fig. 1 and convert it to kinetic plots to find a linear relationship in the corresponding order (i.e., zero, half, or first-order) of kinetics. Attempts to determine a Q_{10} for the entire reaction are similarly flawed because of the implicit linearity assumption. (Examples of these approaches can be seen in Labuza and Bergquist, 1983 and Tazi et al., 2009). The glaring problem with this approach is that it is impossible to fit a linear line to curve containing both linear and exponential areas (Fig. 2). Fitting a straight line through the entire data region of Fig. 1 (both lag and exponential phases) is simply inaccurate and useless. Fitting a straight line through the exponential region of Fig. 1 merely yields the rate of rancidity development and does not predict lag phase and thus shelf life. Furthermore, many oxidative reactions are characterized by both monomolecular and bimolecular reactions (Labuza and Dugan, 1971; Labuza and Bergquist, 1983; Ortolá et al., 1998) and a single line cannot be fit to both reaction regions when this occurs. For example, lipid hydroperoxides will both form and decompose during lipid oxidation reactions so unique approaches are needed to model these reactions (Aragao et al., 2008). Researchers should use great caution when trying to summarize kinetic reactions. Unfortunately, the literature does not always reflect such caution resulting in misinterpretation of results. Overall, more accurate shelf life predictions could be made if the kinetics of chemical reactions could be made before fatty acid oxidation and thus rancidity development begins, e.g., during the lag phase. This could be done by measuring the kinetics of antioxidant degradation or free radical generation and may provide a mechanism to predict the end of the lag phase. This could be particularly important in low-moisture foods those have long shelf-lives, and thus these methods could greatly reduce the time necessary to predict shelf-life.

EFFECT OF WATER AND a_W ON LIPID OXIDATION

Water activity strongly impacts reactions be they enzymatic, non-enzymatic browning, lipid oxidation, or microbial

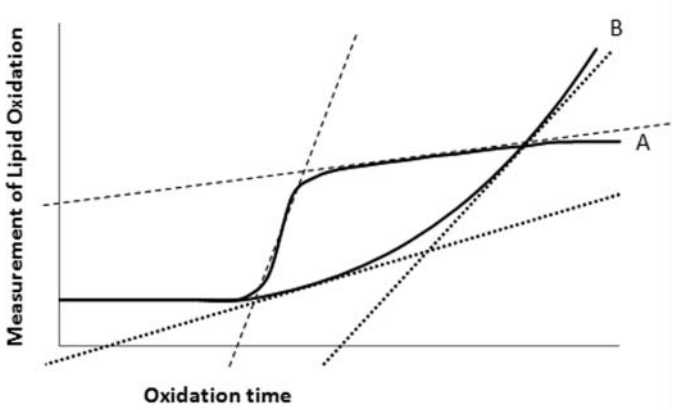

Figure 2 Fitting straight lines through oxidation data does not accurately assess reaction rates because no single line fits through all data. Dashed lines correspond to line A (from Fig. 2), while dotted lines correspond to line A.

growth. Microbial growth generally increases with increasing a_w. For many other chemical reactions, a decrease below $a_w = 1.0$ can initially cause an increase in reaction rates as substrates and reactants become more concentrated, but a further decrease in a_w will significantly decrease reaction rates because most reactions become diffusion-limited. Using microcrystalline cellulose and amylopectin models, Chou et al. (1973) found that a_w primarily affects matrix swelling and thus substrate/reaction site availability as well as catalyst mobility. More recent studies of powders (Kwok et al., 2010) and solid-state pharmaceuticals (Waterman et al., 2012) have reached the same conclusions. Most food systems exhibit hysteresis in sorption isotherms such that the rate of reaction at a given a_w depends on whether the system is gaining (absorption) or losing (desorption) water, with desorption rates always being greater than absorption rates, as first observed by Labuza and Dugan (1971) and Chou et al. (1973). Researchers commonly create these sorption curves because real foods tend to show greater lipid stability at one a_w than another. Overall stability is governed by minimizing multiple deleterious reactions and preventing structural damage. However, in freeze-dried beef, both lipid oxidation and protein solubility must be maintained to ensure quality and these processes are affected by a_w differently, and protein solubility can even influence lipid oxidation reactions (Sun et al., 2002).

Monolayer Theory

Early studies on the lipid oxidation of freeze-dried microcrystalline cellulose models and freeze-dried salmon led to a proposal that lipid oxidation is unique among reactions because the reaction rate increases at very low a_w (Maloney et al., 1966; Martinez and Labuza, 1968; Labuza et al., 1970; Labuza and Dugan, 1971; Chou et al., 1973). For example, lipid oxidation is significantly faster in peanuts stored at water activities of 0.19 vs. 0.60 (Reed et al., 2002). Based on observations in specific food products, it has been suggested that a monolayer of water—or rather, the water saturation of polar groups in lipids—is necessary to cover the surface of the lipid, preventing it from direct exposure to air. This monolayer is essentially "bound" water with limited mobility and is assumed to not participate in chemical reactions, serve as an aqueous-phase reaction medium, or freeze/evaporate as readily (Almasi, 1978). Poole and Finney (1983) found that the saturation of the charged and polar groups, i.e., the monolayer, was the lowest a_w that allowed for chain rotation and conformational changes of proteins in dried egg white. Several studies have found that a variety of foods (Iglesias and Chirife, 1976) such as most spices (Marcos et al., 1997) and peanut flakes (Hill and Rizvi, 1982) are most stable to lipid oxidation at a relative humidity or a_w consistent with the monolayer—most commonly calculated through the empirical two-parameter BET (Brunauer–Emmet–Teller) or three-parameter GAB (Guggenheim–Andersen–de Boer) models for isotherms.

The problem with this monolayer theory, however, is that it cannot be applied universally; even the researchers who promote this theory have found conflicting evidence. For instance, Martinez and Labuza (1968) found that the primary lipid oxidation products in freeze-dried salmon actually decrease as relative humidity increases above the monolayer content; the authors concluded that lipid stability and pigment stability are actually greatest in freeze-dried salmon at intermediate moisture contents. Other foods that are most stable at an a_w above their respective monolayers are illustrated in Fig. 3. Finally, the monolayer value is not exact. Its value is temperature-dependent such that increased temperatures are associated with decreased monolayer values for a single food product, and the differences between values cannot be explained by the thermal expansion of water alone (Iglesias and Chirife, 1976; Almasi, 1978). Furthermore, calculation of the value relies on a number of simplifications and assumptions. Despite the dated research on monolayer applicability, researchers today still find it an easy way of reporting supposed stability. Thus, the monolayer concept is illustrative of trends in lipid oxidation but is highly empirical and applicable only in some circumstances.

Glass Transition Theory

Because a_w fails to universally predict lipid stability, others argue that food stability should be evaluated using glass transition concepts, particularly in foods whose stability is characterized by textural defects like stickiness, structural collapse, and/or loss of crunchiness (Sun et al., 1996; Rahman, 2009; Ergun et al., 2010). Research consistently shows that water added to a glass will have a plasticizing effect, thereby decreasing the glass transition temperature (T_g), stability of the food, and shelf life (Slade and Levine, 1991). However, it is important to note that most foods have only glassy *regions* and are not homogenous glasses (Peleg, 1992, 1996). By using

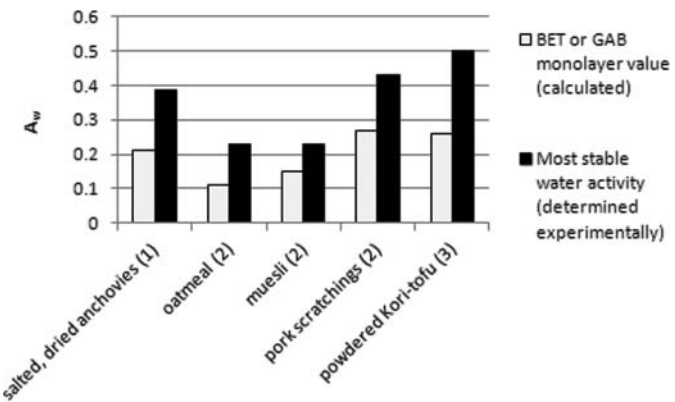

Figure 3 Comparison between monolayer values and the lowest water activity value for greatest stability. Sources: (1) Han et al. (1973); (2) Jensen and Risbo (2007); and (3) Homma and Fujimaki (1982).

electron spin resonance in a freeze-dried emulsion with stripped rapeseed oil, Orlien et al. (2000) found that the glassy matrix effectively trapped hydrophilic radicals and prevented their detrimental reactivity. However, radicals in the non-glassy oil phase still promoted hydroperoxide formation, and oxygen was also able to diffuse, albeit slowly, through the glassy matrix.

Stability at the glassy region differs markedly from that of low a_w (e.g., stability at the monolayer). The principle behind the stability of glassy regions is that, again, water is limited in mobility, therefore leading to slow degradative reactions. However, in glassy regions, mobility is limited by the high viscosity of the product rather than the a_w (Roos and Karel, 1991); rapid changes in temperature—rather than a_w—induce the changes that are associated with transitions to a glass (Peleg, 1996). Furthermore, a_w concepts assume a product is at thermodynamic equilibrium with its environment, whereas the glassy state is a kinetically metastable state. Thus, some researchers claim that the glass transition theory is more applicable to real-world foods, notably those containing protein and/or saccharide polymers (Slade and Levine, 1991; Roos, 1993). This difference in applicability likely explains why water activity and glass transition concepts often yield different predictions for product stability in complex foods. For instance, one research group measured monolayer values (fitting with both BET and GAB) and the critical water activity required to reach the glass transition point (defined by dynamic oscillation on a rheometer) at three different temperatures in freeze-dried abalone (Sablani et al., 2004) and shark (Sablani and Kasapis, 2006). Regardless of the source of freeze-dried muscle or testing temperature, they found the glass transition approach predicted stable foods at an a_w around 0.5, whereas the monolayer approach predicted stable foods at a lower a_w near 0.2. Their conclusions were not, however, correlated to sensory data or actual measurements of lipid oxidation. In another muscle study, Rahman et al. (2009) compared moisture contents in freeze-dried grouper to lipid oxidation (peroxide values) and concluded that the glass transition (measured using differential scanning calorimetry) concept better described stability than the monolayer concept. Differences between the applicability and validity of the glassy state and a_w concepts have been extensively reviewed elsewhere (Bell, 1994; Kasapis, 2005; Abbas et al., 2010; Jacob et al., 2010). In other cases, water activity simply is not a relevant parameter for dictating stability. Whole milk powder, for instance, typically has a water activity near 0.2, but rancidity remains problematic. One group stored whole milk powder ($a_w = 0.23$) at temperatures just above (55°C) and below (37 and 45°C) its glass transition temperature ($T_g = 48$°C) and followed lipid oxidation by measuring pentanal and 2-heptanone (via gas chromatography) and free radical generation (via electron spin resonance) (Thomsen et al., 2005). The authors found that lipid oxidation was not affected by changes in lactose crystallization, a process found to affect a_w but not the total moisture content.

As with the monolayer value, glass transition temperatures are typically not exact and are dependent upon the experimental parameters and conditions under which they are defined (Hutchinson, 2009) and will likely vary depending on whether calorimetry or rheological methods are used. Furthermore, the concept of infinite stability below the T_g is not always true. In the aforementioned milk powder study, for instance, Thomsen et al. (2005) found that lipid oxidation increased directly with temperature, following zero-order kinetics. Milk powder stored 3°C below the T_g still exhibited significant increases in free radicals and both secondary and β-scission oxidation products. Most problematic, however, is improper use of the kinetic models for studying regional glass transitions. Specifically, the kinetics—and their respective models—vary for a glassy state vs. the same material that is transitioning to a glassy state or that is fully plasticized (Peleg, 1996). The other major problem is that many researchers have accepted "universal" constants for the WLF (William, Landel, Ferry) equation, and these constants are too generalized—and therefore invalid—across material types and temperatures (Peleg, 1992).

The Role of Water in Lipid Oxidation—Other Hypotheses

The above discussion shows that water plays both protective and prooxidative roles in lipid oxidation. In some foods at low a_w near the monolayer moisture content, water is protective, presumably because it provides a barrier between the lipid and oxygen. While the classic food stability map proposed by Labuza et al. (1972) shows lipid oxidation having a U-shaped relationship to a_w, studies on freeze-dried emulsions (Ponginebbi et al., 2000) and freeze-dried beef at 60 days (but not always at earlier time points; Sun et al., 2002) showed lipid oxidation increased only at low water activities. In other words, no U-shape was observed; lipid oxidation actually slowed as a_w increased. The authors concluded that increasing a_w caused structural collapse in the freeze-dried foods, thereby eliminating pores and decreasing oxygen exposure. The effect of pores in food will be discussed in greater detail in an upcoming section on coatings. While this finding may minimize lipid oxidation, the structural collapse means the resulting food product is still of unacceptably poor quality.

Water may also be protective because it hydrogen-bonds to lipid hydroperoxides, thereby increasing their relative stability (Labuza et al., 1970; Karel and Heidelbaugh, 1973). The hypothesis of more stable water-peroxide complexes is supported by Chen et al. (1992), who found that increasing the concentration of water from 0 to 2% slowed the decomposition of methyl linoleate hydroperoxides. The authors argued there is a finite number of binding sites, which explains why the greatest improvements in stability occurred between 0 and 1% water concentration. Water was also found to complex with metal ions such that increasing the water concentration to 2% decreased the rate of hydroperoxide decomposition in the presence of 100 ppm Co^{2+} (Chen et al., 1992).

In contrast, water can be detrimental primarily because it solubilizes certain prooxidants like metal ions. Further complicating matters, researchers suspect water influences the formation of lipid oxidation secondary products (Prabhakar and Amla, 1978). When studying carbonyl formation in walnut oil, the researchers found the proportion of different classes (ketoglycerides vs. saturated aldehydes, etc.) varied directly with water activity in oxidized samples. The effect was not observed, however, in samples with low hydroperoxide concentrations (i.e., fresh). The authors hypothesized this effect on secondary oxidation products may explain why some products are stable at water activities about their monolayer values. To our knowledge, no similar recent studies were conducted.

Overall, monolayer and glass transition concepts might not effectively predict lipid oxidation reactions in some foods if oxidation is primarily occurring in the lipid phase and thus would not be significantly impacted by water and the physical state of proteins and carbohydrates. On the other hand, water can play a major role in lipid oxidation chemistry if reactions are primarily promoted by water-soluble prooxidants such as metals. Unfortunately, the causes of lipid oxidation in low-moisture foods are poorly understood, which could be why measurements of water activity, monolayers, and glass transitions do not consistently predict lipid oxidation kinetics.

ABATEMENT AND REDUCTION STRATEGIES FOR LOW-MOISTURE FOODS

Low-moisture foods include dried and dehydrated foods, powdered beverages, chocolate, nuts, and many baked products like crackers and ready-to-eat cereal. While the composition and processing operations for these foods vary greatly, some commonalities remain among the strategies to reduce lipid oxidation.

Reformulated Products and Controlled Processing Conditions

Monitoring the quality of lipid ingredients is the first step in increasing shelf life as poor quality fats high in lipid hydroperoxides will lead to rapid oxidation. Ingredients containing high amounts of prooxidants could also promote lipid oxidation. Ingredients that could be a source of transition metals (e.g., iron), lipoxygenases, and photosensitizers (e.g., riboflavin and chlorophyll) should be avoided if possible. Destroying the physical assembly of plant structures could release prooxidative lipases and lipoxygenases. Finally, any ingredients that alter a_w may also have a measurable impact on lipid oxidation.

An additional technique to decrease oxidation is for manufacturers to reformulate products to be higher in saturated fats as they are more stable to oxidation. However, such a substitution goes against nutritional recommendations (U.S. Dept. of Health and Human Services, 2010). One might think that using fat mimetics—commonly made of carbohydrates, polysaccharides, and/or proteins—will reduce lipid oxidation if lipids are totally replaced. However, even very small amounts of lipids can cause rancidity since sensory detection thresholds for lipid oxidation products can be very low. Even the small amounts of naturally occurring phospholipids in raw ingredients can cause rancidity, a problem that is seen in nonfat dried milk and whey powder (Bassette and Keeney, 1960; Karagül-Yüceer et al., 2002). Despite such complexities, simple ingredient substitution is occasionally quite effective. For instance, Lin et al. (1998) found that lipid oxidation of extruded dry pet food was a function of fat type (poultry fat oxidized faster than beef tallow), although they also found, surprisingly, that fat-free control treatments oxidized faster than their fat-containing counterparts.

Food manufacturers can also modify processing conditions to help control lipid oxidation reactions. Given that lipid oxidation rates increase as a function of temperatures, processing temperature appears to be an obvious target. For instance, supercritical fluid extrusion occurs at lower temperatures than traditional extrusion, and it has been shown to both improved vitamin retention and reduced lipid oxidation in whole grain puffed rice (Paraman et al., 2012). Similarly, vacuum frying has been shown to preserve carotenoids and ascorbic acid content in fried potatoes, apples, and carrot chips (Dueik and Bouchon, 2011) because the lower temperatures needed for vacuum processing prevent heat degradation to the endogenous antioxidants. However, advantages to using high temperatures do exist. Vegetables are typically blanched before freezing because the heat deactivates the aforementioned problematic enzymes (lipase and lipoxygenase). Furthermore, high temperatures associated with processing are important for eliminating pathogens and generating characteristic flavors. Thus, reducing processing temperature may be difficult and undesirable from other, non-oxidative, quality viewpoints. Fortunately, some of the Maillard browning products generated by processing temperatures can act as antioxidants, as was found in fried carrot, potato, and apple chips (Dueik and Bouchon, 2011). Bressa et al. (1996) found that the Maillard products generated during the baking of butter cookies were antioxidative against peroxyl radicals, although Trolox, a vitamin E derivative, was 20 times more effective per gram basis. Similarly, Borrelli et al. (2003) found melanoidins isolated from the surface of bakery products were not toxic to Caco-2 cells and were able to inhibit certain enzyme reductases and transferases. Thus, Maillard browning products are not purposefully added as antioxidant ingredients but are an inevitable consequence of processing parameters involving high temperatures. Interested readers should consult Rosario and Francisco (2005), who provide an excellent review on the interactions between Maillard browning and lipid oxidation pathways and products, a topic beyond the scope of this article.

Like temperature, minimizing oxygen should be another target for reducing lipid oxidation in low-moisture foods. Processing operations that increase oxygen exposure or change

the physical structure of the food can both affect the lag period duration. Oxidative degradation of endogenous antioxidants like carotenoids and ascorbic acid is minimized by drying vegetables under nitrogen (vs. air), as was shown in potatoes (2% less loss), carrots (15% less loss), and paprika (13% less loss) (Ramesh et al., 1999). Mixing parameters can further affect lipid oxidation. For example, Caponio et al. (2008) found that significant lipid oxidation occurred during the kneading step of Italian biscuit (cookie) manufacture. The resulting secondary oxidation products generated during kneading were volatilized during baking so that quality was not immediately compromised. However, identifying this step as a key oxidation point may allow manufacturers to adjust their methods since any processes that promote oxidation will lead to the destruction of antioxidants even if they do not immediately degrade sensory properties. If processing destroys antioxidants then the shelf life of the product will likely be decreased. For example, in the aforementioned biscuit manufacturing (Caponio et al., 2008), perhaps kneading times could be reduced, temperature elevation by kneading could be decreased, or controlled atmospheric processing conditions used to decrease oxidative stress and ultimately increase shelf life. Lin et al. (1998) found that lipid oxidation rates in extruded dry pet food could be controlled by altering the feed moisture content (low-moisture oxidized faster).

Changing processing parameters may be effective (or detrimental) because they ultimately change the product microstructure. For instance, Desobry et al. (1997) blended β-carotene with maltodextrin and dried the emulsion by spray, drum, and freeze driers. Drum drying caused the greatest initial loss in β-carotene but ultimately produced the most stable product, which had lower surface carotenoids and larger particle size (smaller surface area). Lin et al. (1998) found that pet food extruded at 300 rpm had a significantly higher lipid oxidation rate than the treatments extruded at 200 and 400 rpm. The researchers concluded that the extrusion rate affects extrudate expansion and that products with a higher degree of expansion are more likely to have larger cells and thinner cell walls, thereby increasing oxygen exposure and making them more susceptible to oxidation. King and Chen (1998) reached a similar conclusion when comparing beef and pork dried by freeze drying vs. vacuum dehydration. Freeze drying produced dried meat with greater surface area, i.e., greater exposure to oxygen. Their conclusion that beef and pork are better dried by low-temperature vacuum dehydration was supported by the observation that myoglobin degradation, a factor that increases the prooxidative activity of myoglobin, was greater in the more porous freeze-dried products. These problems of surface area are further discussed in the next section on coatings.

Ultimately, reformulating products and altering processing parameters may be difficult for manufacturers who are looking to maintain sensory quality while increasing throughput, automation, and efficiency for as little cost as possible, and optimization is clearly empirical and case-dependent.

Coatings

Oxidation at the high oxygen environment of food surfaces can be especially problematic, which suggests that a coating creating an oxygen impermeable barrier between the food and air would improve shelf life. For example, commercially available nuts are traditionally subjected to thermal treatment to improve microbial safety and digestibility, in addition to enhancing color and flavor. Such thermal treatments may include frying in oil and dry roasting, with both methods generating lipid oxidation products not found in raw nuts. Commercially prepared fried nuts oxidize primarily on the surface, which suggests that (1) the quality of the frying oil may greatly impact overall stability during storage, (2) frying may compromise the physical protection of only the outermost layer of the nut, and (3) oxygen only penetrates the outmost layer of the nuts preventing oxidation on the interior lipids. This latter case would suggest that surface oxidation may be the determining factor of shelf life of nuts (Marmesat et al., 2006). Even in dry-roasted nuts, surface oxidation is the determining factor because endogenous oil migrates to the nut surface and interacts with atmospheric oxygen (Lin and Krochta, 2006; Wambura and Yang, 2010). As expected, ground coffee oxidizes faster than whole coffee beans under ambient conditions (Baesso et al., 1990).

Surface oxidation could be merely at the outermost geometries of a sample, or it could be any place where the food has direct contact with air, be it enclosed air bubbles or cracks at the surface of the product. For example, starch extrudates—which also contained linoleic acid—with glassy regions have a shorter shelf life than do rubbery-state extrudates, and it is assumed the difference is due to microscopic cracks at the surface of the glassy regions (Gray et al., 2008). This is similar to the findings by Lin et al. (1998) on extruded pet food and by King and Chen (1998) on freeze-dried meat—two studies mentioned in the previous section regarding processing effects on microscopic structure.

Coatings, like packaging technologies (see below), may function by influencing oxygen and moisture parameters in foods. If surface exposure is indeed the greatest contributor to lipid oxidation, then the exposure could be minimized to decrease lipid oxidation. In nature, antioxidant-rich skins encase the edible parts, and the exterior shells may also provide some oxidative protection in seeds and nuts (Lou et al., 2004; Rodrigues et al., 2006; Pinheiro do Prado et al., 2009), but such protection is obviously lost when the nut/seed is processed for consumption. For example, unmilled oats are much more oxidatively stable than dehulled oats (Girardet and Webster, 2011). Researchers have developed coatings from a wide array of materials. For instance, antioxidant-rich prickly pear syrup has been used to coat peanuts and reduce oxidation during roasting and storage (Mestrallet et al., 2009). Colzato et al. (2011) found that coating macadamia nuts with edible, zein-based films reduced lipid oxidation in the unshelled nuts because the hydrophobic films decreased permeation of

oxygen. Zein, whey protein, and carboxymethylcellulose have also been used successfully to reduce lipid oxidation in roasted peanuts, although the coatings were most effective when the peanuts were first sonicated to remove surface lipids, presumably improving coating attachment and decreasing the concentration of oxidizable lipids at the surface (Wambura and Yang, 2010). The removal of surface lipids might be difficult at the industrial level, however, since practices like ultrasonication are typically reserved for benchtop studies.

Protein and polysaccharide coatings may have the advantage of minimally altering the food's native texture and flavor. Alternatively, the confectionary industry uses chocolate and compound coatings as moisture and oxygen barriers. Spray drying is another means of coating because emulsified active ingredients are typically encased in either a protective glassy or crystalline shell of carrier material like maltodextrin or sugar. Spray drying is most effective if the active ingredients are successfully encased by the wall material because PUFAs at the surface of spray-dried particles oxidize faster than internal PUFAs (Desobry et al., 1997). Optimizing the carrier material can, however, significantly improve encasement efficiency or change pore size. Vega and Roos (2006), e.g., provide an excellent review of how encasement efficiency has been maximized in dairy-like products. Rather than using whey protein for a carrier, for instance, it is now common to use sodium caseinate because it is a better emulsifier and more readily resists heat denaturation. As noted in the review, sodium caseinate is even more effective when used in a 1:1 ratio with lactose because rapid drying of the sugar enhances glass formation. Pore size is important in spray-dried powders because it limits the access of oxygen to the encapsulated materials. Research has shown that the addition of mono and disaccharides to the maltodextrin carrier will improve oxidative stability of the powder by reducing pore size and slowing oxygen diffusion (Desobry et al., 1999). While decreasing pore size can potentially decrease oxidation rates, one should also realize that the formation of the glassy regions by maltodextrins during spray and freeze drying can trap oxygen within the particle core, and this oxygen can be sufficient to promote oxidation (Andersen et al., 2000). This is especially true with lipids such as omega-3 fatty acids whose oxidation products have extremely low sensory perception levels. Furthermore, oxygen is diffusible, albeit at very slow rates, through the glassy matrix (Orlien et al., 2000). The type of carrier used will affect oxygen's ease of diffusion and rate of lipid autooxidation. Drusch et al. (2009) reached that conclusion by using carbohydrate carriers of varying molecular weights and dextrose equivalents to dry an emulsion with fish oil. Positron annihilation lifetime spectroscopy revealed that differences in free volume elements existed among the carriers, even though they controlled the viscosity of the feed emulsion, oil droplet size, and particle size, density, and surface area. Nevertheless, the protective coating afforded to liquid emulsions by powdering technologies represents a key preservation method for food, cosmetic, and pharmaceutical applications. One need only search for "dried emulsions" to find any number of articles varying carrier material, drying parameters, use of agglomeration, and, more often, the emulsion itself via interface structure (multi vs. single-layered), interface type (proteins vs. carbohydrates), interfacial charge and charge density, oil source, and other variables. The end concept remains the same: encapsulating oil offers protection from lipid oxidation, but this is not a magic bullet as additional antioxidant strategies are often also need to obtain maximum stability.

Finally, saturated fat may even be used as a protective coating, such as when it is sprayed on the surface of pet foods (Clark, 2004). As previously explained, fats are more susceptible to oxidation as the number of unsaturated bonds increases, so saturated fats are inherently more stable. The quantity of saturated fat sprayed on the surface will affect nutrition claims, as well as the wettability of spray-dried powders, so as with all approaches, care must be given to consider the food system and its purpose.

Antioxidant Addition

Arguably the most common approach manufacturers use to control oxidation is to add antioxidants directly to products like crackers, chips, cereal, and spray-dried emulsions, i.e., foods not naturally rich in antioxidants and/or high in endogenous prooxidants. Antioxidants are those compounds that, at a particular concentration, inhibit oxidation. Regardless of whether the antioxidant is synthetic or naturally derived, common mechanisms of action exist. These include free radical scavenging, metal chelating, and singlet oxygen quenching. Excellent reviews of antioxidant mechanisms are found in any number of external sources (Madhavi et al., 1996; McClements and Decker, 2008; Nanditha and Prabhasankar, 2009).

Despite an array of mechanisms and types of antioxidants, numerous variables like polarity, the food matrix, side reactions, volatility, and the presence of regenerating and/or synergistic antioxidants all affect antioxidant effectiveness. Furthermore, there is a move for "natural" antioxidants like vitamin E (α-tocopherol), vitamin C (ascorbic acid), carotenoids (β-carotene and vitamin A), and plant phenolics as many manufacturers strive for their products to contain only "words you can pronounce" or "ingredients found in the kitchen." Unfortunately, that leaves manufacturers with fewer and, in some cases, less effective options. For instance, researchers found that the synthetic antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were more effective at preventing oxidation in a methyl linoleate model system over a range of temperatures than were α-tocopherol or isopropyl citrate (Ragnarsson et al., 1977). In contrast, ferulic acid and sodium phytate were used to successfully replace BHA in sugar snap cookies without any loss in shelf life or sensory characteristics (Hix et al., 1997). Rosemary extract was more effective than either mixed tocopherols or TBHQ

(tertiary butylhydroquinone) in extruded jerky-style salmon snacks (Kong et al., 2011). Natural phenolics in meat are suspected to reduce lipid oxidation in extruded, ground meats (Rhee et al., 1999). Viscidi et al. (2004) found benzoin, chlorogenic acid, and quercetin all reduced hexanal formation up to 12 weeks in extruded oat cereals, but only quercetin was effective beyond 12 weeks, keeping hexanal formation low up to 24 weeks. Unfortunately, 24–46% of the added phenolics were lost during the extrusion process, making these antioxidants potentially expensive ingredients to add. As can be seen by the lack of consistent antioxidant performance in the above samples, the best antioxidant for each food usually needs to be evaluated on a case-by-case basis. This is because free radical scavenging activity predicted by in vitro assays often does not relate to antioxidant activity in food (Alamed et al., 2009), presumably because so many factors can impact antioxidant activity in complex food systems. For example, the effectiveness of an antioxidant can be influenced by its physical location within the food (is it at the site of oxidation?), survival during food processing operations, and interactions with other food components such as other antioxidants and prooxidants.

Due to labeling laws, high purification costs, and/or an inability to pinpoint the most effective, single antioxidant compound, manufacturers may often use whole foods and extracts to help control oxidation. For example, rosemary leaves and/or rosemary extract—appearing as natural flavoring on the ingredient label—are commonly added to ground turkey. Extracts from mangoes, bananas, guava, and other fruits have been proposed as antioxidant sources for used in baked products due to their high polyphenol content (Nanditha and Prabhasankar, 2009). Green tea extract containing any number of phenols and/or catechins also appears commonly on labels. Grape seed extract, often used as an antioxidant in meat systems, has recently been found to extend the shelf life of extruded corn chips (Rababah et al., 2011). Wheat bran has been found to have scavenging activity for hydroxyl radicals, making it an ideal antioxidant for use in cereal products (Martínez-Tomé et al., 2004). Klensporf and Jelen (2008) found that inclusion of defatted red raspberry seed extract in dry muesli cereal decreased hexanal formation by 29% in an 11-day period. The phenolics and flavonoids in hemp powder have been shown to reduce oxidation in extruded rice bars when hemp powder was incorporated at 20% (Norajit et al., 2011). Unfortunately, many of these natural antioxidant sources require high concentrations to be effective or are not commercially available.

How antioxidants are applied to low-moisture foods can be important because antioxidants are more likely to be diffusion-limited than they are in bulk oil or food dispersions. It is, unfortunately, difficult to find studies that systematically compare antioxidant inclusion at different locations in low-moisture foods. However, one study on fried crackers containing fish oil found it was more effective to add TBHQ to the frying oil rather than the cracker dough (Ahmad and Augustin, 1985). Other studies found it better to add antioxidants to the surface of fried foods because the protective species were too volatile in the hot frying oil. For example, Sharma et al. (1997) found sprinkling BHA, BHT, and TBHQ at 2% (w/w) directly on the surface of fried potato and banana chips immediately after frying significantly improved shelf life at 37°C with TBHQ and BHA being over twice as effective as BHT.

Packaging

Packaging follows processing before product distribution and can be used to provide light and air barrier properties, incorporate antioxidants, or control storage atmospheres to reduce lipid oxidation rates. In low-moisture foods, most packaging contains laminates that minimize moisture migration, and a number of materials can be used such things as thermoplastic, petrochemical polymer resins (e.g., polyethylene terephthalate, polypropylene, polyvinyl chloride, etc.), aluminum foil, and bioplastics (using starch or cellulose or biopolymers from soy, corn, peas, and other bio-degradable materials) (Coles and Kirwan, 2011). Like everything else, package selection requires optimization. Linoleic acid in commercial corn flour is better protected from lipid oxidation when stored in pouches of ethyl vinyl alcohol rather than pouches of polyethylene film (Márquez-Castillo and Vidal-Quintanar, 2011). The authors attributed the results to differences in oxygen-barrier properties. Recent development of a film derived from fish proteins was shown to effectively reduce oxidation in dried fish powder (Artharn et al., 2009). Specifically, round scad protein-based film containing 25% palm oil (to reduce water vapor permeability) and 40% chitosan (to improve mechanical properties and reduce oxygen transfer) was found to minimize formation of thiobarbituric acid reactive substances in samples of dried fish powder covered with film better than the control (no film), fish protein film without chitosan, fish protein film without palm oil, or high-density polyethylene film. The authors attributed this reduced lipid oxidation to the film's low permeability to oxygen.

Antioxidants may be added to packaging materials to either inhibit degradation of the polymer packaging itself or to preserve the packaged foodstuff. In the latter case, antioxidants are typically released from the packaging because they either volatilize or because moisture uptake causes packaging degradation and subsequent release of antioxidant (Anderson and Shive, 1997). Polyethylene, polypropylene, and polyvinyl chloride packaging all show the capability to hold and release antioxidants (Dopico-García et al., 2007), and this has helped prolong the shelf life of crackers and breakfast cereals (van Aardt et al., 2007). The protective effects likely depend on both the polymer and antioxidants used. For instance, low-density polyethylene film with BHT prolonged the shelf life of oatmeal, but α-tocopherol offered no protective benefits presumably due to its low volatility (Wessling,

2001). Recently, poly (lactic acid) films (PLA) have garnered attention for their ability to incorporate and release bioactive compounds and antioxidants (Manzanarez-López et al., 2011; Soto-Valdez, 2011; Iñiguez-Franco et al., 2012). For instance, Ortiz-Vazquez et al. (2011) found that BHT, when added to PLA films, would release into its surrounding environment and more readily migrate toward oil than either ethanol or water. The authors concluded "PLA-BHT functional membranes have the most potential for release of antioxidant in non-water environments." Poly(lactide-co-glycolide) films loaded with either 2% α-tocopherol or 1% BHT + 1% BHA have also been shown to extend the shelf life of whole milk and buttermilk powders. Authors speculate that the mechanism is by direct antioxidant contact with milk fat since there was not enough moisture in the dried systems for hydrolytic degradation of the packaging to occur to release the antioxidant (van Aardt et al., 2007). A common trend in all articles is that large quantities of the antioxidant are lost during polymer extrusion and/or the storage process, making the process somewhat costly. However, adding antioxidants to the packaging allows manufacturers to reduce the load of antioxidants added directly to the product, and problems with volatilization can be remedied by adding excess antioxidant, as was done for oatmeal stored in high-density polyethylene film with BHT (Miltz et al., 1988). Finally, it should be noted that other researchers have developed packaging with iron-chelators, but this has not yet been tested with low-moisture foods (Tian et al., 2012). Numerous reviews (such as Shin and Lee, 2003; Lopez-Rubio et al., 2004) discuss other active packaging strategies that may not necessarily be applied to low-moisture foods. A disadvantage of some active packaging is that it may cause undesired changes in bulk properties or the polymer (flexibility, transparency, etc.) may be difficult to store while also maintaining activity (e.g., antioxidant activity), and may be subject to regulatory issues (Goddard et al., 2012).

The goal of modified or controlled atmospheric packaging is to minimize oxygen concentration or control the humidity to achieve a moisture content corresponding to maximum product stability. Unfortunately, results are often mixed. For instance, packaging peeled almonds in nitrogen-reduced conjugated diene formation, but the same results were not achieved with roasted almonds. In addition, nitrogen atmosphere had no effect on hydroperoxide formation or sensory scores in either roasted or peeled almonds (Sanchez-Bel et al., 2011). On the other hand, Scussel et al. (2011) found that packaging shelled Brazil nuts in ozone both improved consumer sensory scores and increased aflatoxin degradation. Storage under vacuum, carbon dioxide, and nitrogen all extended the oxidative stability of commercial corn flour compared to the control, but carbon dioxide and nitrogen exhibited the most promising effects during the 160-day study at 55°C (Márquez-Castillo and Vidal-Quintanar, 2011). No statistical difference in lipid oxidation existed between freeze-dried beef stored in vacuum packaging vs. ambient air, however (Sun et al., 2002). Vacuum packaging effectively prevents staling. A sealed bag at 156 days has the same oxidative values as an opened bag at 64 days (Baesso et al., 1990).

Oxygen absorbers in "active" packaging have effectively reduced hexanal generation in hermetically sealed tin crackers (Berenzon and Saguy, 1998). The packaging for many snack products is often flushed with nitrogen gas with the assumption that limiting oxygen will increase shelf life. The results vary, however, with some studies showing varied degrees of success, while others show no statistical difference between the inert gas and oxygen (Paik et al., 1994; Del Nobile, 2001). The effectiveness of flushed packaging is, of course, dependent on the barrier properties of the packaging itself and the efficiency of producing a hermetic seal after packing. Unfortunately, some studies do not take care to separate these convolutions. The inconsistency in these results may be attributed to other factors as well, particularly the presence of atmospheric oxygen. First, the product itself contains air. This is especially true of products like crackers that rely on air pockets to achieve the desired texture. Second, to our knowledge, no studies have examined the efficiency of nitrogen flushing. It is probable that the air in the corner of a bag of chips, e.g., is not entirely flushed out due to packaging dimensions (corners are difficult to reach) and hindrance of food (some chip may block the corner from being flushed). Third, while pure nitrogen is inert, the nitrogen gas used for flushing typically contains some oxygen. Manufacturers of nitrogen gas generators for the food industry advertise that different products produce nitrogen gas in a purity range from 95 to 99.999%, with respective escalating costs. One can therefore imagine that pure nitrogen is rarely used in food manufacturing and packaging. Unfortunately, all three of the aforementioned scenarios introduce or maintain oxygen in the food product or packaging, and essentially any amount of oxygen is sufficient for promoting lipid oxidation because of the exponential nature of the reaction.

FUTURE RESEARCH NEEDED TO DEVELOP IMPROVED ANTIOXIDANT TECHNOLOGIES FOR LOW-MOISTURE FOODS

Most of the original research on lipid oxidation in low-moisture foods has focused on impact of water activity and evaluation of different antioxidant technologies. While the work on water activity provides some insight into lipid oxidation mechanisms in low-moisture food, there are probably many other factors that also impact lipid oxidation pathways and mechanisms. For example, what are the major prooxidants and reactive oxygen species; what is the location of the lipids and in particular the lipids most susceptible to oxidation? How do antioxidants partition in low-moisture food? Without more knowledge of the mechanisms of lipid oxidation in low-moisture foods, it will continue to be impossible to rationally develop new antioxidant technologies.

Low-moisture foods such as baked goods present some unique physical and chemical environments that likely impact chemical reactions. Like all foods, the surface of baked goods in contact with air could be the site of oxidation. However, baked goods often contain some type of leavening agent. The presence of a leavening agent—in addition to mechanical mixing and kneading during processing—will ultimately lead to air bubble development. These leavening agents could impact oxidation as they can alter pH, an important factor in prooxidant metal reactivity and antioxidant effectiveness. In addition, air is hydrophobic so it is possible that these endogenous air bubbles affect partitioning of lipids. If lipids associate with air bubbles, is this interface a site of oxidation? How will the solid fat content of lipids added to baked goods impact their partitioning? Would liquid oils be in different locations than solid lipids, and could this effect their interactions with air interfaces? Another trait of low-moisture foods is that they are made from flours and these flours contain different amounts of lipids. All purpose and whole wheat flour contains 1.0 and 2.5% lipids, respectively. Whole wheat flour is known to be more susceptible to rancidity development. However, little is known about how the location, physical properties, and composition of these endogenous lipids impact the oxidation of baked goods. For example, after milling, could they be high in lipid hydroperoxides and free fatty acids, which could promote oxidation? Do they contain antioxidants that contribute to the oxidative stability of baked goods? The role of the proteins in flours (e.g., glutens) in lipid oxidation is also not known. Since proteins can be surface active, could they be associated with the lipid fraction. If they are, could they impart a charge to the lipid interface that would attract or repel prooxidant metals they could alter oxidation rates? Glutens are also high in sulfhydryls and other free radical scavenging amino acids and, as with other proteins, could chelate metals. Could these properties inhibit oxidation, and could they be manipulated to further change oxidation pathways?

Flour in low-moisture foods also introduces other complications in the form of prooxidants. For instance, flour naturally contains iron and all-purpose flour is typically fortified with iron. Lipid oxidation in rice crackers containing only glutinous rice flour and water has already been attributed to endogenous iron (Maisuthisakul et al., 2007). Only trace amounts (<50 ppb) of iron are needed to decompose hydroperoxides to free radicals (Decker and McClements, 2001; Waraho et al., 2011), so eliminating the iron from food is generally not feasible. Questions that need to be answered to better understand the role of iron as a prooxidant in baked goods includes: Do both endogenous and added iron promote oxidation; where does the iron partition in baked goods; does the iron interact with other baked good components in a manner that decreases (proteins) or increases (reducing agents) their reactivity. Baked goods could also contain photosensitizers (e.g., riboflavin) that can produce singlet oxygen in the presence of light. However, very little is known if these photosensitizers are active in low-moisture foods. All-purpose flour is also subjected to other treatments; to our knowledge, no studies have looked at the effect of flour bleaching agents on lipid oxidation in the baked goods. These bleaching agents could oxidize the endogenous lipids, antioxidants, and proteins resulting in an increase in oxidation (e.g., via formation of lipid hydroperoxides or via the destruction of tocopherols and antioxidative sulfhydryls on proteins).

It is well documented that the physical properties of colloidal lipids impact oxidation chemistry. It is possible that lipids could be encapsulated into different structures that increase their oxidative stability and then be added for baking. For instance, encapsulated lipids could be engineered to have low interfacial permeability (e.g., multilayer emulsions or hydrogels) to decrease interactions between the lipids and prooxidants in the flour and/or, have a positive interfacial charge to repel prooxidative metals (reviews in Coupland and McClements, 1996; Waraho et al., 2011). Furthermore, emulsions can be spray-dried or freeze-dried into powders to create a glassy carbohydrate layer that is impermeable to oxygen. Some anecdotal evidence suggests that encapsulated lipid can be stable in low-moisture foods (e.g., omega-3 oils in bread). However, for the encapsulated lipids to be effective, their structures must survive during the processing and storage of the low-moisture foods. Very little is actually known about which encapsulation methods would be most effective and which conditions are ideal to preserve the integrity of the encapsulation in low-moisture foods.

Finally, researchers need to understand the behavior of antioxidant in low-moisture foods. It is generally agreed that antioxidants are most effective when they partition into the lipids most susceptible to oxidation. Antioxidant partitioning has been shown to vary with the polarity of the antioxidant (e.g., increasing partitioning into the lipid with increasing antioxidant hydrophobicity). In some food systems (e.g., emulsions), antioxidants that partition at the oil–water interface are often more effective (Panya et al., 2012). In addition, antioxidant partitioning can also be impacted by pH, which can alter the charge of the antioxidant and other food components such as proteins, which can bind phenolic compounds. To our knowledge, nothing has been published on the partitioning behavior of antioxidants in products such as baked goods.

Most low-moisture foods are thermally heated, often by baking or extrusion. This can cause loss of antioxidants by thermal destruction, increasing oxidation rates, and causing antioxidant volatilization. Thermal processing could also change antioxidant partitioning as the lipid melts and re-solidifies as the crystallization of lipids can often cause the expulsion of minor lipid components (Berton-Carabin et al., 2013). Additional questions that need clarification to better understand lipid oxidation in low-moisture foods include: Does a lack of moisture prevent diffusion of antioxidants to the site of lipid oxidation? Does oxidation occur at the surface of a baked good meaning that topical application of antioxidants could be more effective than inclusion in the initial formation?

NEW TECHNOLOGIES TO STUDY LIPID OXIDATION IN COMPLEX FOODS

The above discussion highlights the need to gain a better understanding of lipid and antioxidant oxidation mechanisms in low-moisture foods. Lipid oxidation is traditionally measured by monitoring the formation of primary (e.g., conjugated dienes and lipid hydroperoxides) and volatile secondary oxidation products that negatively impact flavor. Barriuso et al. (2013) provide an excellent review of traditional methodologies for assessing lipid oxidation in all food systems, as well as improvements made upon those methodologies within the last decade. However, these methods alone cannot provide all the information needed to learn more about lipid and antioxidant oxidation mechanisms in foods. A major limitation of these methods is that they cannot predict the kinetic end of the lag period. As previously mentioned in the discussion on lag phases, new methods are needed to measure oxidation in the early stages of storage, before the product fails sensory analysis. A potential technique for monitoring free radicals in the early stages of storage is electron paramagnetic resonance (see below). Also, since antioxidants preserve foods by being oxidized before fatty acids, the loss of antioxidants during storage could provide information on oxidative processes in the lag phase and which antioxidants are at the site of lipid oxidation. Both of these techniques could also provide information that could be used to predict the length of the lag phase potentially providing information that could be used to predict shelf life.

New methods are also needed to determine how the physical properties of foods impact lipid oxidation chemistry. Such physical properties are likely key to understanding the unique oxidation patterns of low-moisture foods vs. bulk oils and liquid emulsions. Researchers are now beginning to employ fluorescent dyes/probes, electron spin probes, and Raman spectroscopy to understand lipid oxidation. Fluorescent dyes are now available that increase or decrease in fluorescent due to oxidative reactions. BODIPY 581/591 is a probe commonly used in biological studies and its decay has, e.g., been related to the concentration of peroxyl radicals in lipophilic solutions and liposomes (Naguib, 1998). Since this particular dye also undergoes shifts in emission wavelength upon oxidation, Pap et al. (1999) used it to visualize and quantify oxidation in cellular membranes. However, care must be taken when interpreting results. For instance, BODIPY 581/591 is sensitive to hydroxyl radicals but not to reducing metals or superoxide anion (Drummen et al., 2002). Furthermore, researchers fear interactions with some antioxidants and have found that the dye overestimates lipid oxidation due to its high susceptibility to oxidation (Itoh et al., 2007; MacDonald et al., 2007). Researchers recently used new dyes, they argue, that allow for real-time and continuous in in vitro and in vivo studies. Changes in the fluorescent intensity of these dyes have already been used to directly assess the barrier properties of structured delivery particles (Tikekar and Nitin, 2011; Tikekar et al., 2011a,b; Li et al., 2013; Mosca et al., 2013).

The above fluorometric probes could also be used in conjunction with other probes in confocal microscopy. Thus researchers could use multiple probes to simultaneously visualize lipids and proteins. The lasers used in confocal scanning light microscopy (CLSM) are strong enough to penetrate thick samples such that the researcher need not worry about slicing delicately thin samples—a tremendous boon since many low-moisture foods are crumbly, fracture easily, or rely on air pockets to contribute to their inherent texture. Furthermore, raster scans are used to image a single plane of the specimen (z-stacks) and then compiled into three-dimensional reconstructions that can elucidate large structures like air bubbles. Unlike scanning and transmission electron microscopy, CSLM requires no extreme sample preparation or analysis in non-native environments that may damage the sample's natural intrinsic structure. Using a combination of confocal fluorescent dyes can provide important information about the physical location of lipids in low-moisture foods (Fig. 4). Oxidatively sensitive fluorescent probes can then be used to determine which lipid populations are most susceptible to oxidation. In addition, antioxidants that naturally fluoresce (e.g., rosmariniac acid) can be used to determine antioxidant locations. The combination of these two probes can provide extremely important information for determining which antioxidant would partition into the oxidizing lipids and thus increasing antioxidant effectiveness.

Another in situ method gaining popularity in food research is electron paramagnetic resonance (EPR) spectroscopy, which

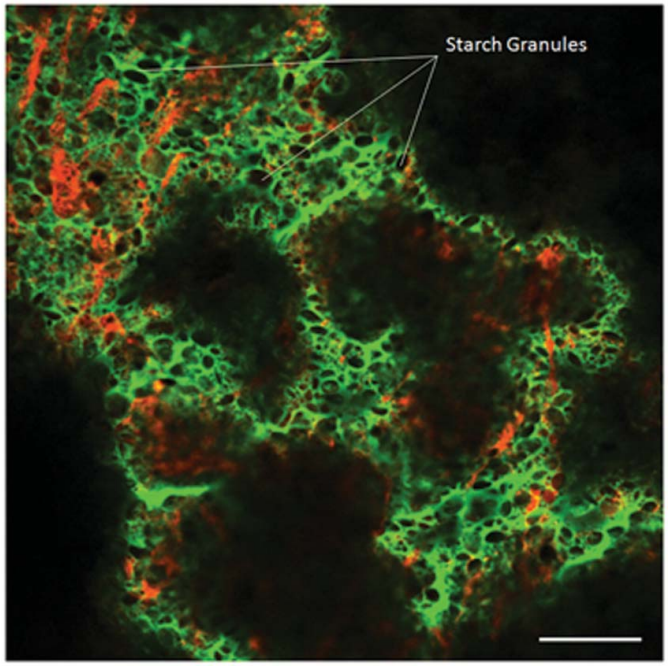

Figure 4 Model cracker imaged on confocal microscope. Green areas represent lipid; red areas represent protein. Scale bar is 50 μm.

measures changes in energy levels of unpaired electrons when they are subjected to magnetic fields, typically from microwave radiation (Andersen and Skibsted 2002). These unpaired electrons have paramagnetic properties and are typically free radicals. Highly stable free radicals called spin probes are commonly added during experiments to measure radical quenching and molecular mobility, and unstable endogenous radicals can be quantified using a technique called spin trapping. Because EPR is sensitive to free radicals, it can be used to assess the earliest stages of lipid oxidation—even earlier than the development of hydroperoxides, which are typically quantified as a primary oxidation products (Andersen and Skibsted, 2002). EPR and spin traps have even been used to detect previously unidentified hydroxyl and sulfite radicals in oxidized wine and also provided the first evidence of the Fenton reaction in wine suggesting that these techniques might also be applicable in low-moisture foods (Elias et al., 2009). This type of spectroscopy has proven useful for lipid analysis in numerous food systems, including tracking light-induced oxidation in cream cheese (Westermann et al., 2009) and measuring oxygen permeation in foods with glassy regions (Andersen et al., 2000; Orlien et al., 2000). EPR may be particularly suited for low-moisture foods as the radicals are likely to be more stable and should not require the spin-trapping technique that has been criticized for introducing foreign substances and potentially promoting side redox reactions (Andersen and Skibsted, 2002). Stapelfeldt et al. (1997) found direct correlations between EPR concentration of free radicals, traditional lipid oxidation measurements (2-thiobarbituric acid reactive substances), and sensory scores in whole milk powders and have recommended EPR for monitoring lipid quality in that product.

Surface-enhanced Raman spectroscopy (SERS) is another technique that can be used to both quantitate and visualize lipid oxidation product directly in foods. SERS combines conventional Raman spectroscopy and nanotechnology. Placement of the sample of interest on noble metal nanoscale-roughened surfaces (typically silver or gold) enhances the inherently weak Raman molecular signatures tremendously because of the large electromagnetic field induced by the excitation of the localized surface plasmon resonance (Haynes et al., 2005). SERS has rapidly developed into a powerful new analytical tool due to its extremely high sensitivity (down to the single molecule level in some cases; Haynes et al., 2005), ability to measure multiple oxidation products simultaneously, and ability to generate two-dimensional maps of the physical location of chemical changes in complex matrices. However, the development and application of this technique for studying lipid oxidation is only now being evaluated. The power and higher sensitivity (100 times higher than that of high pressure liquid chromatography (HPLC) fluorescence) of SERS was clearly demonstrated in a proof-of-concept study by Zhang et al. (2010), who quantified adducts of thiobarbituric acid and malondialdehyde with silver nanoparticles. As previously mentioned, such high sensitivity means SERS could provide more information on oxidative processes earlier in the shelf life of foods thus providing important information during the lag phase.

While the aforementioned methods have elucidated key information, they, too, fail to answer *all* of the questions of the previous section. Thus, our understanding of lipid oxidation in low-moisture food may advance only with the development of even more new analyses.

CONCLUSION

Although lipid oxidation is a chemical process, it can be influenced by both chemical (e.g., antioxidant inclusion) and physical (e.g., formation of crystalline regions) parameters. Consumer demand for more "natural" and "clean" labels has limited the development of new, synthetic antioxidants as well as use of currently approved synthetic antioxidants in low-moisture foods, leaving manufacturers with few options. This has recently become even more of a challenge for the food industry due to the removal of hydrogenated oils and the desire to improve the nutritional profile of their products by adding more PUFAs. Therefore, there is a clear need for systematic research studying mechanisms of lipid oxidation in low-moisture foods since a better understanding of oxidative pathways will allow for the development of technologies to improve shelf life. Such research is key to reducing consumers' saturated fat intake by developing healthful products high in unsaturated fats that meet expectations for high quality and long shelf lives.

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