CHAPTER 1: INTRODUCTION

Current Strategies to Prevent Oxidation in Foods

Oxidation is one of the most serious problems the food industry faces in protecting shelf-stable foods due to its deteriorating effects on food quality. The major food quality issues influenced by lipid oxidation include decreased nutritional quality, increased toxicity, development of off-odor, and altered texture and color (Finley and Given, 1986). The initial products of autoxidation are hydroperoxides, which are colorless, tasteless, and odorless. However, these hydroperoxides decompose to low-molecular-weight compounds that result in the development of rancid flavors and odors. Research also suggests that oxidation of lipids from the diet may play a direct role in the development of chronic diseases in the human body (Riemersma, 2002; Kanazawa and others 2002). Several food additive ingredients and food packaging strategies are currently employed to prevent these harmful oxidative reactions within food systems.

Synthetic (e.g. BHA, BHT) and natural antioxidants (e.g. ascorbic acid, tocopherols) are added directly to foods as primary antioxidants, which donate hydrogen atoms to quench peroxyl radicals before they can further react with unsaturated lipids. Phenolic antioxidants form a radical with low reactivity, due to delocalization of the unpaired electron over the aromatic ring, and exhibit no further potential to react with lipids after hydrogen abstraction. Antioxidants significantly extend the shelf life of foods containing lipids susceptible to oxidation such as vegetable oils, animal fats, flavorings, spices, nuts, processed meats and snack products. The need for antioxidants is not limited to high-lipid foods, but also includes products such as cereals which contain only 2-5% lipid components. Synthetic antioxidants also are released from cereal box liner bags followed by sorption into the food, which provides oxidative protection. Chelating agents, such as EDTA, are added to foods as secondary antioxidants to bind trace metals, which act as prooxidants.

Traditional glass and metal packaging are considered excellent barriers to oxygen. Polymer packaging also can be used to limit ingress of oxygen. High barrier films, such as ethylene vinyl alcohol, aluminum metallization and, most recently, clay nanocomposites have been used to reduce oxygen permeability. Similar to aluminum metallization, deposition of a thin layer of low permeability silicon oxide on a thermoplastic substrate has been used to greatly improve barrier
properties. The oxygen permeability of these laminated films is very low relative to most polymer films, but still several orders of magnitude higher than that of silica glass.

Oxygen is removed from some foods, such as ground coffee by vacuum packaging. Package atmospheres have been modified by flushing with inert nitrogen in products such as chips and nuts. Iron-based oxygen scavenging sachets are used for their ability to preferentially oxidize, resulting in removal of oxygen from the package. Polymer-based oxygen scavenging films absorb oxygen in modified atmosphere packages and also serve as a total oxygen barrier when activated. This polymer oxygen scavenging system remains inactive until the scavenging reaction is triggered with UV light.

Oxidatively susceptible products, such as beer and wine, have traditionally been packaged in pigmented glass bottles. Pigmented high density polyethylene (HDPE) has been used in milk packaging to limit the degree of product photooxidation. UV light absorbers have been added to polymer packaging of fruit beverages to allow delivery of 100% of the recommended daily allowance of vitamin C without the ascorbic acid experiencing the typical oxidation to dehydroascorbic acid.

**Oxidation Remains Problematic**

Oxidation remains a problem to the food industry because of some rather obvious factors including the difficulty in removing environmental oxygen completely from foods and the value consumers place on viewing the product through the package. It remains very difficult to remove the entire headspace oxygen and that dissolved or trapped within the beverage or food matrix. While vacuum packaging is effective, it cannot be used with many foods and a high-barrier film also must be employed or its benefits will not be realized. Consumers continue to value the clarity of the package, but this also allows visible and/or UV light to impact the product and serve as an initiator of the oxidative reaction.

Initiation is used to form the low concentration of radical required to start the chain reaction. Propagation describes the chain reaction itself and termination is the process by which radical centers are quenched. Because oxidation is a chain reaction process, an extremely proactive approach must be taken to prevent or limit the initiation step. Oxidative problems may also persist because antioxidants in foods are completely consumed by free radicals before the labeled expiration date of the product.
The continual push of food companies to extend product shelf life has allowed greater periods of time for the ingress of oxygen. Many of the current food packaging polymers are poor oxygen barriers, such as HDPE used in milk product packaging. Large volumes of particulate foods, such as powdered milk, remain particularly susceptible to oxidation during storage due to their extremely high surface area. Diets rich in unsaturated fats also have been touted to consumers for their health benefits and, therefore, it is likely that more products susceptible to oxidation will reach the marketplace. For example, the replacement of partially hydrogenated oils in snack foods with more unsaturated oils, such as soybean and olive, presents new challenges due to increased problems of oxidation. New technologies that are more efficient and have greater capacity to prevent radical initiation and hydroperoxide decomposition are still needed by the industry to solve the oxidation problem.

**Active Food Packaging Technologies**

Food and beverage packaging has been traditionally defined as a passive barrier to delay the adverse effect of the environment on the contained product. However, current research trends include the development of packaging materials that interact with the environment and with the food, playing an active role in preservation. Active packaging is primarily designed to prolong shelf life, improve safety and/or enhance sensory properties in foods and beverages. Intelligent packaging contains indicators such as time-temperature, gas atmosphere, microbial growth, and pathogen detection and is used for quality control of packaged food.

Active, controlled, and intelligent packaging systems have experienced explosive growth in the United States. Countries outside the U.S. are well-positioned as leaders in these packaging technologies with a forecasted 11.3% average annual growth rate through 2008 (Business Communications Company, 2004). During the past decade, active packaging has experienced significant growth and change as innovations have challenged the status quo of traditional food and beverage packaging. Food polymer packaging can serve as a reservoir or matrix from which active ingredients are delivered in a controlled manner into the product. The controlled release of active ingredients is one such innovation that can be categorized within the active packaging field. Antimicrobial preservative releasers, antioxidant releasers, and flavoring and aroma emitters are examples of active packaging systems for preservation and shelf life extension of foods or improving their quality.
The worldwide market for active, controlled, and intelligent packaging of foods and beverages is expected to grow to an estimated $134 billion by 2008 (Business Communications Company, 2004). As technological packaging innovations progress and material costs decrease, food and beverage manufacturers will begin to implement controlled release technology into their packaging. Advances in controlled release packaging technology allow food and beverage manufacturers to improve flavor and aroma delivery, delay the onset of lipid oxidation, and inhibit pathogenic and spoilage microorganisms in their products. While controlled release active packaging is not currently in wide commercial use, it is poised to improve the safety and enrich the quality of the global food supply.

In the 1960s, the modern view of drug delivery began in which the effectiveness of drug performance was increased by specific targeting and timed delivery. These technologies allowed greater control of the therapeutic concentration for a longer duration than conventional dosing. Significant research on the incorporation of active compounds within food packaging and its subsequent controlled release to the packaged product began in earnest in the 1990s. The use of synthetic polymers as food packaging materials has increased enormously during the past decades due to their advantages over other traditional materials, such as metal, glass, and paperboard. Controlled release packaging is well-suited for controlling continuous food degradation reactions, such as microbial growth and lipid oxidation, because constant replenishment of active compounds can maintain safety and quality. Food packaging materials may be incorporated with cyclodextrins to change the release rate of active compounds. Cyclodextrins can be considered as empty capsules of molecular size that form inclusion complexes with guest molecules, resulting in an encapsulation process on the molecular scale. Cyclodextrin inclusion complexes have been formed with many active ingredients including antioxidants, antimicrobials, and flavor and aroma compounds.

Compounds with antioxidant activity may exhibit prooxidant action under certain conditions (Takahashi, 1995; Lankin and others 1999). Natural antioxidants, including phenolic antioxidants and tocopherols, have been shown to undergo loss of activity and become prooxidants at high concentrations (Jadhav and others 1996). The direct addition of antioxidants to food in one large initial dose is limited by the potential for rapid depletion of the antioxidants, in addition to very high initial concentrations. Modern consumer trends show increasing concern with the use of synthetic chemicals and the belief that natural antioxidants are safer and of
greater nutritional benefit. Therefore, a need exists in the food industry to develop polymer packaging which can deliver natural antioxidants in a controlled manner throughout the product shelf life.

Research Objectives

This research aims to initiate a discourse on the mass transfer mechanism of a natural antioxidant additive within a polymer matrix and its subsequent delivery to the surface of a solid food product, thereby contributing an antioxidant effect to the food system. The accomplishment of the following research objectives will allow another packaging innovation to be available to food and packaging technologists, which may be used in a hurdle approach to inhibit oxidative processes. The controlled release of natural antioxidants from polymer packaging may more effectively limit oxidation, maintain nutritional quality, inhibit the formation of harmful oxidation products, and extend the shelf life of foods.

Objective 1: Optimize the cyclodextrin complexation methods of the natural antioxidants, α-tocopherol and quercetin, and characterize their cyclodextrin inclusion complexes.

Objective 2: Incorporate cyclodextrin inclusion complexes of α-tocopherol and quercetin into linear low density polyethylene films while maintaining their relative antioxidant activity and stability.

Objective 3: Measure the release kinetics of α-tocopherol and quercetin in their free and cyclodextrin inclusion complexed forms from linear low density polyethylene films into a coconut oil model system.

Objective 4: Monitor the primary and secondary oxidation products of a tocopherol-stripped corn oil model system in the presence of controlled-release antioxidant packaging films.
References


CHAPTER 2: REVIEW OF LITERATURE

Antioxidants function to intercept and react with free radicals at a rate faster than their typical substrates. These free radicals have the ability to attack various targets, including lipids and proteins, and initiate oxidative reactions that result in the decreased shelf life of many foods. Antioxidants can play a preventative role through H-atom transfer in the lipid peroxidation pathway:

\[
\begin{align*}
\text{Initiation} & : \quad RH \longrightarrow R^* \quad (1) \\
\text{Addition of O}_2 & : \quad R^* + O_2 \longrightarrow RO_2^* \quad (2) \\
\text{H-atom exchange} & : \quad RO_2^* + RH \longrightarrow ROOH + R^* \quad (3)
\end{align*}
\]

Reactions 2 and 3 form a chain reaction when a free radical R• has been generated. Many lipid molecules (RH) are converted into lipid hydroperoxides (ROOH) as the chain cycles through reactions 2 and 3, resulting in oxidative rancidity of lipids. The reaction rate of 2 is very fast at about \(10^9 \text{ M}^{-1}\text{s}^{-1}\), while the rate of 3 is much slower, generally \(10^1 \text{ M}^{-1}\text{s}^{-1}\) (Wright and others 2001). Phenolic antioxidants (ArOH) contain at least one hydroxyl group attached to a benzene ring and have the role of interrupting the chain reaction as follows:

\[
RO_2^* + ArOH \longrightarrow ROOH + Ar^* \quad (4)
\]

The phenolic antioxidant must be a relatively stable free radical to be effective, so that it reacts slowly with substrate RH but rapidly with RO_2^*.

**α-Tocopherol**

α-Tocopherol (Figure 2.1) is a chain-breaking antioxidant that reacts with peroxyl radicals with a rate constant of about \(10^6 \text{ M}^{-1}\text{s}^{-1}\), which is much faster than the reaction of peroxyl radicals with lipid RH (Wright and others, 2001). The bond dissociation enthalpy (BDE) of phenolic antioxidants is an important factor in determining the antioxidant effectiveness, since the reaction rate with free radicals is faster with weak OH bonds. The RO_2^* radical has a BDE on formation of the parent ROOH of about 88 kcal/mol, which will react rapidly in an exothermic reaction with α-tocopherol. The BDE of α-tocopherol is about 76 kcal/mol, while β-
, $\gamma$-, and $\delta$-tocopherol have slightly higher respective BDEs of 78, 78, and 80 kcal/mol (Wright and others, 2001). These BDEs enable the tocopherols to function as effective chain-breaking antioxidants that prevent lipid peroxidation. The rate constant for the hydrogen atom transfer from $\alpha$-tocopherol to cumylperoxyl radicals at 25 °C decreases by approximately 2 orders of magnitude upon transitioning from hexane to ethyl acetate as solvent (Valgimigli and others 1999). This solvent effect may be explained in terms of hydrogen bonding between $\alpha$-tocopherol, which acts as hydrogen donor, and the solvent, which acts as hydrogen acceptor (Pedrielli and others 2001). The more solvating solvent induces added stability to the reactants relative to the transition state, which effectively increases the activation energy of the reaction.

Most vegetable oils contain tocopherols with the more unsaturated oils having higher concentrations of up to 1000 mg/kg or greater (Rossell, 1991). In the more saturated vegetable oils, such as coconut and palm kernel, tocopherols are almost completely lacking. Several types of tocopherol exist such as the $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-forms, which differ from one another in the position and number of methyl groups on the phenol ring. A series of corresponding tocotrienols also exists in which the 16-carbon side chain is unsaturated. During the storage of unsaturated oils, tocopherols are consumed and their concentrations fall as oxidation proceeds. In coconut oil, $\alpha$-tocopherol concentrations have been reported in the range 0.9–5 mg/kg (Slover, 1971; Rao and Perkins, 1972; U.S. Department of Agriculture, 2007). $\alpha$-Tocopherol exhibits the highest antioxidant activity of the tocopherols in vegetable oil with the least stability during storage (Player and others 2006). The degradation rate of $\alpha$-tocopherol for 10 days of storage in soybean oil was 5.6% per day. $\alpha$-Tocopherol degraded faster than both $\gamma$- and $\delta$-tocopherol with its degradation rate 10 times faster than $\delta$-tocopherol. $\alpha$-Tocopherol easily donates a hydrogen atom to the peroxyl radical due to its relatively low BDE and is the most easily destroyed. $\alpha$-Tocopherol is expected to function as a more potent hydrogen donor than $\gamma$- or $\delta$-tocopherol due to its fully methylated structure (Player and others, 2006).

The reported stability of $\alpha$-tocopherol varies widely among researchers and appears strongly dependent upon factors of atmosphere, sample matrix, and analytical method. The stability of pure $\alpha$-tocopherol upon storage was approximately halved with each 10 °C increase in temperature (Lips, 1957). The half-life of pure $\alpha$-tocopherol at a temperature of 40 °C was 113 days, while at 120 °C the half-life was reduced to only 0.9 days. The degradation of $\alpha$-
tocopherol followed an induction type of curve with $\alpha$-tocopherol having less stability in its pure form than when diluted in an oxidatively stable solvent. A complete loss of $\alpha$-tocopherol is observed upon storage of red palm oil, groundnut oil, and their mixed blends (Lakshmi and Sarojini, 1996). $\alpha$-Tocopherol is highly unstable to heat in red palm oil with 89% losses due to heating at 130 °C for 15 min during a frying process (Lakshmi and Sarojini, 1996). Significant decreases in tocopherols also occurred during the extrusion process of fish- and peanut-containing half-products (Suknark and others 2001). Decreases in $\alpha$-tocopherol content in fish and peanut extrudate were 23 and 18%, respectively, under extrusion conditions of around 100 °C and 250 rpm. These differences in $\alpha$-tocopherol content are attributed to differences in fatty acid composition of the raw materials. Oxidative degradation of $\alpha$-tocopherol has been studied by microcalorimetry and is clearly a temperature-dependent process between 50–80 °C (Otsuka and others 1994).

Thermal oxidation of $\alpha$-tocopherol at 200 °C resulted in $\alpha$-tocopherylquinone as a degradation product (Chung, 2004). Both tocopherol and tocopherylquinone were further degraded into fragments primarily at non-aromatic parts. The degradation products of $\alpha$-tocopherol were combined with tocopherylquinone to produce thermal products through a dimerization process. Under an inert atmosphere that completely excludes oxygen, no degradation of $\alpha$-tocopherol was observed at temperatures up to 240 °C, but the presence of any trace of oxygen will result in its immediate oxidation (Verleyen and others 2001). In monoacid triacylglycerols, $\alpha$-tocopherol losses of nearly 10% per hour were observed at 180 °C, leading to complete depletion after 10 h of heating (Barrera-Arellano and others 1999).

**Quercetin**

Flavonoids are a group of aromatic secondary plant metabolites that belong to a class of phenolic substances. The major classes of flavonoids include flavones, flavonols, flavanones, isoflavones, anthocyanidins, and cathecins. Quercetin (Figure 2.1) is the most common flavonol found in the human diet and epidemiological studies suggest it protects against cardiovascular disease (Erlund, 2004). The predominant sources of quercetin in the diet include onions, apples, tea, and berries. Quercetin has been reported to be among the most effective of the flavonoid antioxidants and the most active in its own flavonol class (Vinson and others 1995). The
The antioxidant activity of quercetin has been evaluated in terms of chemical structure-activity relationships. Flavonoids with the same basic structure, such as quercetin, rutin, and apigenin, have peroxyl radical scavenging activity that increases with the number of –OH substituents (Cao and others 1997; Silva and others 2002). Three structural determinants are proposed to be responsible for effective radical scavenging by flavonoids: (1) the ortho-catechol group in the B-ring, which gives high stability to the radical formed; (2) the conjugation of the B-ring to the 4-oxo group by the 2,3-double bond that ensures electron delocalization from the B-ring; and (3) the 3- and 5-OH groups with the 4-oxo group, which allows electrons to be delocalized from the 4-oxo group to both substituents (Bors and Saran, 1987). These structural features function together to enable a higher electron delocalization which gives a higher stability to aroxyl radicals.

Quercetin is a potent antioxidant in oil systems due to its free radical scavenging activity, UV-absorbing characteristics, and iron-chelating capacity (Chen and Ahn, 1998). Quercetin shows greater antioxidant effectiveness against UV-induced lipid oxidation in an oil emulsion than BHT (butylated hydroxytoluene). Quercetin did not have significant antioxidant activity in sunflower oil at 60 °C in the presence of endogenous tocopherols and added citric acid (Roedig-Penman and Gordon, 1998). A significant antioxidant effect is found with the presence of tocopherols alone, indicating quercetin is active as a metal-chelating agent in this system. Quercetin exerts strong antioxidant effects in fish oil and a synergistic effect when combined with α-tocopherol (Nieto and others 1993).

Oxidative degradation of quercetin is observed to cause unusual changes in antioxidant capacity, which is both temperature and solvent dependent (Pinelo and others 2004). Different solvent systems appear to select the specific reaction pathway that is followed. In ethanol or methanol, oxidative reactions result in the formation of complex polymers, which lead to an initial increase and then subsequent decrease in antiradical activity. In hydro-alcoholic solutions, a decrease in antiradical activity is observed during quercetin degradation. The polymerization of quercetin may be prevented by the presence of water with its high hydrogen-accepting capability, which makes oxidative cleavage the most probable reaction pathway.

Quercetin experiences degradative losses of approximately 83% in 0.2 M KOH solution at pH 13.0 and 97 °C within 30 min (Makris and Rossiter, 2000). Degradation of quercetin under these strong alkaline conditions occurs at a slower rate at 20 °C, but results in nearly complete
decomposition within 250 min. Oxidative conditions convert flavonols into compounds that are sensitive to degradation even at ambient temperature. The presence of oxygen in these quercetin solutions greatly accelerated its degradation rate with complete degradation occurring within only a 40 min period at 25 °C.

Quercetin is able to retard the oxidation of methyl linoleate in the hydrogen-bond-accepting solvent of tert-butyl alcohol, although at a much slower rate than α-tocopherol (Pedrielli and others, 2001). The antioxidant efficiency of quercetin has been estimated by comparison of its inhibition rate constants with that for α-tocopherol under the same experimental conditions. Quercetin is 30 times less effective than α-tocopherol in inactivating peroxyl radicals in tert-butyl alcohol. The value of the inhibition rate constant of quercetin is similar to those measured under the same experimental conditions for the synthetic antioxidants BHT and BHA (butylated hydroxyanisole). The antioxidant mechanism of quercetin in a lower hydrogen-bond-accepting solvent appears similar to that of α-tocopherol (Pedrielli and others, 2001). In chlorobenzene, quercetin behaves as a chain-breaking inhibitor as evidenced by a clear induction period, rather than as a retarder. The inhibition rate constant of quercetin in chlorobenzene is about 18 and 4 times more reactive than BHT and BHA, respectively.

**Antioxidants as Prooxidants: A Paradox**

Antioxidants may exhibit prooxidant behavior dependent upon the specific conditions of the system, particularly antioxidant concentration level, sample matrix, and the presence of transition metals. Prooxidant activity can accelerate damage to lipids, proteins, carbohydrates, pigments, and vitamins. Most of the phenolic acids, flavonoids, anthocyanidins, and anthocyanins exhibit some prooxidant activity at low concentrations, while this is not observed with α-tocopherol (Fukumoto and Mazza, 2000). Quercetin prooxidant activity increases with increasing concentration until the antioxidant activity of the compound rapidly becomes dominant at about 200 µM. The prooxidant effect of flavonoids is most pronounced at low antioxidant concentrations (<1–2 µM), but is reversed at higher concentrations and masked by dominant inhibition of initiation (Dangles and others 2000). Flavonoids acted as prooxidants rather than antioxidants in the presence of Cu²⁺, and this activity always increased with flavonoid concentration in the range studied up to 2 µM (Cao and others, 1997). The copper-initiated
prooxidant activity of flavonoids is dependent upon the number of –OH substitutions in the flavonoid structure.

Tocopherol in bulk vegetable oils is observed to experience antioxidant activity inversion with its greatest activity at lower concentrations and either decreases or even prooxidant activity at higher concentrations. The prooxidant effects of α-tocopherol observed at high concentrations are not well understood (Frankel, 1998). At high concentrations, α-tocopherol inhibits hydroperoxide decomposition but promotes hydroperoxide formation (Frankel, 1996). The optimal concentration for α-tocopherol to exhibit greatest antioxidant potency is around 100 ppm in soybean oil (Jung and Min, 1990; Evans and others 2002). Peroxide values of α-tocopherol increase as the α-tocopherol concentration increases from 100 to 1000 ppm with significantly higher values at 500 ppm indicating prooxidant activity (Jung and Min, 1990). α-Tocopherol functions as the most potent of the tocopherols in soybean oil at preventing oxidation as measured by conjugated diene formation (Evans and others, 2002). The maximum antioxidant activity of α-tocopherol in stripped corn oil was determined to be 100 ppm on the basis of hydroperoxide formation (Huang and others 1994). Low concentrations of α-tocopherol in the 50–100 ppm range are optimal to minimize oxidation of mackerel oil with higher concentrations proving less effective (Zuta and others 2007). Oxidized tocopherols are indicated to act as prooxidants in soybean oil, with oxidized α-tocopherol exhibiting a stronger effect than oxidized γ- or δ-tocopherol (Jung and Min, 1992).

Properties of Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides of α-D-glucopyranose formed by the action of certain enzymes on starch. The most common CDs are α-CD, β-CD, and γ-CD, which contain six, seven, and eight glucose units, respectively. Table 2.1 summarizes several of the important properties of α-CD, β-CD, and γ-CD, which are commonly referred to as the native or natural CDs. CDs containing less than six glucose units are too strained to exist and those with greater than eight units are very soluble, hard to isolate, and infrequently studied. The native CDs are crystalline, homogeneous, and nonhygroscopic compounds. The CD molecule is described as a conical cylinder or shallow truncated cone as depicted in Figure 2.2a. The glucose units are linked to each other by glycosidic α-1,4 bonds as illustrated in Figure 2.2b. The CD
can form intramolecular hydrogen bonds between its secondary hydroxyl groups. A complete secondary belt is formed by these hydrogen bonds making β-CD a rather rigid structure. This intramolecular hydrogen bond formation is the most likely explanation for the low water solubility of β-CD relative to the other native CDs. In the α-CD molecule, the hydrogen bond belt is incomplete since one glucose unit is in a distorted position. The γ-CD molecule has a noncoplanar, more flexible structure, which makes it the most soluble of the native CDs (Szejtli, 1998).

The diameter of the CD cavity is larger on the side containing the secondary hydroxyl groups. The side containing the primary hydroxyls has a reduced cavity diameter due to the free rotation of these primary hydroxyls. The nonbonding electron pairs of the glycosidic oxygen bridges are oriented toward the inside of the cavity, which produces a high electron density inside and imparts some Lewis base characteristics (Szejtli, 1998). The environment within the CD cavity has been inferred by some calculational studies. Lichtenthaler and Immel (Lichtenthaler and Immel, 1996) concluded that the three native CDs are very similar with the wider secondary hydroxyl end of the cavity being hydrophilic and the narrower primary hydroxyl end being hydrophobic. The polarity of β-CD in a 10 mM aqueous solution appears to identical with that of a 40% ethanol-water mixture (Szejtli, 1996a). The dielectric constants for γ-CD and β-CD have been estimated as 55 and 48, with the slightly larger effective dielectric constant attributed to the larger γ-CD cavity’s ability to include more water molecules (Street and Acree, 1988).

The unique structural properties of the CD cavity explain some of the atypical characteristics of these molecules. The exterior of the CD molecule has numerous hydroxyl groups and, therefore, is fairly polar. The interior of the cavity is nonpolar relative to the exterior and relative to the common external environments, specifically water (Connors, 1997). CDs act as host molecules to form inclusion complexes fairly nonspecifically with a wide variety of guest molecules. The only apparent requirement is that the guest molecule must fit into the cavity, even if only partially (Saenger and others 1998). The most probable mode of binding, derived from both thermodynamic and NMR studies, involves the insertion of the less polar part of the guest molecule into the cavity. The hydrophilic groups, such as hydroxyl, amino, and carboxyl, or the charged groups, such as ammonium and carboxylate, remain exposed to the bulk solvent even after inclusion of the hydrophobic segment (Rekharsky and Inoue, 1998). Complex stabilities are greater for neutral compounds compared to those of the corresponding charged
species derived from the same original guest molecules. Weak forces that function locally in CD inclusion complexation are not always cooperative in nature and may act independently or even counteract one another in some cases (Rekharsky and Inoue, 1998). The flexibility of the guest molecule has an important role in the stability of CD complexes. Increasing flexibility in a guest molecule leads to a more favorable complexation entropy because there are more possible “conformers” that can fit properly into the cavity (Rekharsky and Inoue, 1998).

The guests in CD complexes are retained within the cavities in the solid state and even in solution at least on a dynamic time scale. The formation of inclusion complexes of guest molecules with CDs in aqueous solution results in a considerable rearrangement and removal of the water molecules originally solvated to both the CD and the guest. This process also initiates the release of water molecules from the CD cavity into the bulk water. The main forces involved in binding are believed to be primarily van der Waals and hydrophobic interactions, but hydrogen bonding and steric effects also can be a factor. In general, the thermodynamic quantities for the 1:1 complexation reactions of natural and modified CDs are consistent with the hydrophobic nature of the host–guest interactions. Connors (1995) compiled the binding constants ($K_{11}$) of 1:1 complexes of $\alpha$-CD, $\beta$-CD, and $\gamma$-CD with many substrates from numerous publications and performed statistical analysis. These complex stabilities appear to be reasonably described as normally distributed in log $K_{11}$ when treated as statistical populations. The mean values of $K_{11}$ for $\alpha$-CD, $\beta$-CD, and $\gamma$-CD are 129 M$^{-1}$, 490 M$^{-1}$, and 355 M$^{-1}$, respectively (Connors, 1995). The efficiency of complexation may sometimes be rather low and, therefore, relatively large amounts of CDs must be used to complex small amounts of drug. Typically, solid drug-CD complexes contain less than 5 to 10% of the guest (Loftsson and Brewster, 1996; Loftsson and others 1999).

Hydration State

The CDs form various stable hydrates that are dependent upon the water vapor pressure of the surrounding atmosphere. Commercially available crystalline $\beta$-CD hydrate is a nonstoichiometric composition which contains 10.5–12 water molecules per CD molecule (Ripmeester, 1993). The water molecules involved may function as space-filling guests enclathrated in the CD cavity. The total water content per $\beta$-CD molecule reduces from about 12.3 at 100% humidity to about 9.4 at 15% humidity (Nakai and others 1986; Steiner and
Koellner, 1994). The \(\gamma\)-CD isotherm shows two phases with the intermediate hydrate containing seven water molecules per \(\gamma\)-CD unit, and the fully hydrated cavity with 17 water molecules per \(\gamma\)-CD at 100\% humidity (Nakai and others, 1986). A neutron diffraction study found 15.7 water molecules in the asymmetric unit of \(\gamma\)-CD that are distributed over 25 positions (Ding and others 1991). A total of 8.8 water molecules are positionally disordered and distributed over 17 positions in the \(\gamma\)-CD cavity.

The water content of \(\beta\)-CD hydrate is in fast equilibrium with ambient humidities, and experiences hydration at an appreciably faster rate than dehydration (da Silva and others 1996). The exchange of water with \(\beta\)-CD and the atmosphere occurs on a minute time scale with dehydration equilibrium being reached in approximately 15 min depending upon the crystal size (Steiner and Koellner, 1994). Both hydration and dehydration equilibration is complete after about 120 min as determined by real-time Raman spectroscopy of \(\beta\)-CD (da Silva and others, 1996).

**Antioxidant Activity**

Uncomplexed \(\alpha\)- and \(\beta\)-CDs have been studied for their direct antioxidant activity and results indicated that 2.3 \(\mu\)M CD provides a protective effect from lipid peroxidation, which was more evident for \(\beta\)-CD (Calabrò and others 2004; Calabrò and others 2005). This effect was presumed to be due to chelation of Fe\(^{2+}\), the inducer of peroxidative damage, by the CD. 10-mM solutions of \(\gamma\)-CD have also shown antioxidative activity under physiological conditions (Tamura and others 1998). In the development of an ORAC assay specific for lipophilic antioxidant activity, Huang and others (2002) reported that randomly methylated \(\beta\)-CD does not possess any antioxidant activity.

CDs can act as secondary antioxidants by enhancing the naturally occurring antioxidant capacity of a food. Secondary antioxidants reduce the rate of oxidation by various processes including binding metal ions, scavenging oxygen, decomposing hydroperoxides to nonradical products, absorbing UV radiation, and deactivating singlet oxygen (Jadhav and others 1996). The capacity of CDs to function as secondary antioxidants in a food system can be evaluated using the system’s protective constant (Núñez-Delicado and others 1997). High values of the protective constant displace the equilibrium to form complexes, which allows natural substrates
to be included in the hydrophobic CD cavity and unavailable to oxidative enzymes. This increases the half-life of natural antioxidants in a food and extends the product shelf life.

**Preparation of Cyclodextrin Inclusion Complexes**

The preparation of CD inclusion complexes is a relatively simple procedure, however, in most cases the reaction conditions must be conformed to the specific guest compound. The majority of the complex formation processes occur in aqueous solutions or at least in the presence of water. The presence of at least a minimum amount of water is necessary for inclusion processes. Cosolvents, such as ethanol, are not required in most cases but are applied in the preparation of CD complexes with drugs and flavors (Szente, 1996b).

In aqueous solutions of CDs or CD derivatives at ambient temperature, the guest is stirred or vigorously agitated until the maximum solubility is attained. In certain cases, additional agitation besides normal stirring, especially ultrasonication, has been reported to increase complexation efficiency. The remaining undissolved guest is removed by filtration or centrifugation, and the clear aqueous solution is evaporated to dryness by vacuum evaporation, spray-drying, or freeze-drying depending on the nature of the guest compound. Complexation in solution is a well-suited method for the highly water-soluble CD derivatives, such as hydroxypropyl-β-CD (HP-β-CD). Several other techniques are used to form CD complexes including: complexation in suspension, kneading, cogrinding or mechanochemical activation, or even melting together the potential guest with CD (Szente, 1996b).

**Solvent Effects**

Cosolvents that show an increase in β-CD solubility rising to a maximum at quite low solvent mole fractions in aqueous solution include tetrahydrofuran, ethanol, and isopropanol (Chatjigakis and others 1992; Donzé and Coleman, 1995). Changes in cosolvent concentration can have strong effects on the relative efficiencies of guest included within β-CD when two guests are present in competition (Donzé and Coleman, 1995). The association constant of complexes decreases with a corresponding increase in the hydrophobicity of the medium, which may be due to increasing the percentage of a certain alcohol or increasing the chain length of a certain alcohol (Junquera and Aicart, 1997). The decrease in association constants is steeper as the number of carbons in the alcohol chain increases. Guest-host associations are canceled with the
addition of a high percentage of alcohol for the short chain alcohols, such as methanol and ethanol. Longer chain alcohols, such as pentanol or even greater length, are able to cancel this association with only 1% of alcohol. A considerable decrease in complexation has been observed in β-CD inclusion complexes with 30% ethanolic water relative to water (Matsui and others 1994). β-CD complexation efficiency can increase with increasing concentrations of ethanol in aqueous solution up to a maximum, beyond which the efficiency decreases (Yoshii and others 1998).

**Benefits of Cyclodextrins**

Complexation of guest compounds with CDs provides certain benefits. Some of these benefits include: alteration of guest solubility, stabilization against the effects of light, heat, and oxidation, masking of unwanted physiological effects, and reduction of volatility (Hedges, 1998). CDs can stabilize, have no effect on reactivity, or accelerate guest degradation. The CD molecule can at least partially shield the guest molecule from attack by various reactive molecules. The most common application of CDs in the pharmaceutical industry is to enhance drug solubility in aqueous solutions. In general, the lower the aqueous solubility of the pure drug, the greater the relative solubility enhancement gained by CD complexation. CDs prove to be useful tools to obtain aqueous drug solutions without the use of organic cosolvents, surfactants, or lipids, and to increase dissolution rates and oral bioavailability of solid drug complexes (Loftsson and Brewster, 1996). The main use of CDs in the food and cosmetic industries is for the molecular encapsulation of flavors and fragrances.

The antioxidant activity of the antitumoral and antibacterial drug, violacein, was enhanced upon complexation with β-CD (De Azevedo and others 2000). Studies on erythrocyte lipid peroxidation by the thiobarbituric acid method showed that 500 µM violacein:β-CD complex inhibited lipid peroxidation completely, while the free violacein showed only 65% inhibition at the same concentration.

**Cyclodextrin Inclusion Complexes of Natural Antioxidants**

CDs have been shown to form inclusion complexes with the several classes of natural antioxidants listed in Figure 2.1. The flavonoid family members, including flavonones, flavonols, and anthocyanins, and α-tocopherol have been reported to form inclusion complexes
with various types of CDs. Many flavonoids form inclusion complexes with β-CD, which could improve the dissolution and following absorption of the antioxidant in therapeutical formulations. The flavonones hesperitin, hesperidin, naringenin, and naringin form 1:1 inclusion complexes with β-CD in solid state and aqueous solution as shown by NMR, FT-IR, DSC and X-ray studies (Ficarra and others 2002).

β-CD and γ-CD were generally found to be the CDs with the most favorable characteristics to obtain inclusion complexes with the flavonols (Bergonzi and others 2000). Inclusion complexes of the flavonols 3-hyrdoxyflavone, morin, and quercetin have been formed with α- and β-CD by the co-evaporation method (Calabrò and others, 2004). Calabró and others (2004) studied the effect of molecular encapsulation on the flavonol’s antioxidant activity by different biological assays. Complexation with both α- and β-CD was shown to improve the antioxidant activity of the flavonols by the bathophenanthroline test, comet assay, and lipid peroxidation. The flavonol antioxidant mechanisms of scavenging free radicals and binding of iron were most improved by β-CD complexation. The protection against oxidative damage provided by complex formation between the glycosylated parent form of quercetin, rutin, and β-CD has also been studied by various in vitro tests (Calabrò and others, 2005). The improved antioxidant activity of the solid 1:1 inclusion complex of rutin with β-CD compared to free rutin is believed to be the result of increased solubility in the biological system.

The anthocyanins callistephen (pelargonidin-3-glucoside) and chrysanthemin (cyanidin-3-glucoside) experienced increasing degrees of color fading in solutions with increasing concentrations of β-CD (Yamada and others 1980). This observation coupled with the reappearance of both anthocyanins’ color when these solutions were strongly acidified support the formation of an inclusion complex. Anthocyanins are susceptible to changes in their chemical structure in weakly acidic aqueous solution which results in the rapid fading into a colorless pseudobase form (Tamura and others, 1998). Yamada and others (1980) suggests that the inclusion complex may protect anthocyanin from decomposition even with its labile pseudobase form. Natural anthocyanidins have a molecular size of approximately 6.6 × 12 Å, therefore, anthocyanins such as naphthalene derivatives are able to fit into the β- and γ-CD cavities (Tamura and others, 1998). The anthocyanins, including four kinds of malvidin glucoside, were found to suppress lipid peroxidation when complexed with γ-CD at
physiological pH (Tamura and others, 1998). The anthocyanin retained its antioxidative activity while complexed with γ-CD and resulted in only a slight reduction in antioxidative power compared to the free anthocyanin.

α-Tocopherol is able to form inclusion complexes with β-CD, HP-β-CD, and hydroxypropyl-γ-CD (HP-γ-CD) of both 1:1 and 1:2 α-tocopherol:CD stoichiometries (Iaconinoto and others 2004). Complexation of α-tocopherol with β-CD and its derivatives does not interfere with the vitamin antioxidant activity (Iaconinoto and others, 2004). The radical scavenging activity of α-tocopherol was measured in vitro using the xanthine/xanthine oxidase enzymatic system and no significant differences were observed between free and CD complexed α-tocopherol (Iaconinoto and others, 2004). Complexation of α-tocopherol with HP-β-CD or HP-γ-CD increased the photo-induced degradation of α-tocopherol, while β-CD complexes did not differ significantly. However, the chemical stability of α-tocopherol was enhanced by complexation with HP-β-CD and HP-γ-CD (Iaconinoto and others, 2004).

The extension of the oxygen radical absorbance capacity (ORAC) assay to measure activity of lipophilic antioxidants has involved the use of CDs as solubility enhancers (Huang and others, 2002; Bangalore and others 2005). Randomly methylated β-CD added at a concentration of 10–40% enhanced the aqueous solubility of lipophilic antioxidants by as much as 1000-fold (Szente and others 1998). The long aliphatic tail of the lipophilic antioxidants, such as α-tocopherol, can fit into the CD cavity during complexation, allowing the hydrophilic phenol group to remain in aqueous solution as a functional antioxidant (Huang and others, 2002).

Analytical Methods of Cyclodextrins and Their Inclusion Complexes

$^{13}C$ CP/MAS NMR

In solution, the fast molecular motion averages the anisotropic chemical shielding to the isotropic chemical shift typically observed. In solids, the lack of molecular motion does not allow averaging of the dipolar interaction, which is the interaction between the magnetic moments of any nuclei with non-zero nuclear spin. The chemical shift is dependent on the orientation of the individual molecule relative to the external magnetic field. Cross polarization (CP) is a technique which achieves heteronuclear decoupling in solids, and results in observed
signal enhancement and faster data accumulation rates. CP experiments are useful when a relatively rare spin such as $^{13}$C is in the presence of a more abundant spin species such as $^1$H.

Magic angle spinning (MAS) is performed by spinning a solid sample at a very rapid frequency about an axis with a 54.7° angle relative to the applied magnetic field. The chemical shielding anisotropy is reduced to a single value at the magic angle, which is an equivalent average value as the chemical shift observed in solution spectroscopy. The MAS technique has the advantage of simplifying spectra by averaging the effects of dipolar coupling and chemical shift anisotropy (Ripmeester and Ratcliffe, 1996). Any differences in isotropic chemical shifts can be attributed to packing effects, hydrogen bonding, or because conformations are locked in the solid state while these are averaged in solution (Ripmeester and Ratcliffe, 1991). The rotational rate to achieve successful MAS is required to be fast relative to the total chemical shift anisotropy before spinning, or on the order of a few kilohertz. If the spinning rate is not fast enough, then spinning sidebands will occur in sample spectra.

In CP/MAS, peak intensity and the ability to detect a signal are dependent upon the selected contact time. Carbon signals result from their interaction with protons through a dipolar coupling. The increase in magnetization of rare-spin nuclei depends on the strength of the dipolar coupling between a particular rare spin and spatially near abundant nuclei. The dipolar interaction is most important for directly bonded nuclei since it is dependent on the inverse cube of internuclear distance, although the interactions are summed over all nuclear pairs in the lattice (Ripmeester and Ratcliffe, 1996). For example, $^{13}$C-$^1$H dipolar coupling is much larger for protonated than for nonprotonated carbons (Hervé and others 1994). The dipolar coupling may be reduced by molecular motion and in free liquids this coupling averages to zero, resulting in no CP. The CP rate indicates the rigidity of the environment with more rapid CP rates for samples having more solid characteristics (Epand and others 2002). Greater signal intensity indicates a higher degree of CP. The signal will decay with a time constant $T_{1p}$, since the magnetization is generated in the presence of a resonant radio frequency field. Spectra obtained with CP cannot be taken as quantitatively correct since certain experimental parameters are not identical for all resonant nuclei in a sample.

The study of $^{13}$C nucleus under conditions of MAS with dipolar decoupling and with $^1$H-$^{13}$C CP has become an important tool in characterizing inclusion compounds in the solid state, especially those compounds with limited solubility. Many methods for the preparation of solid
inclusion complexes involve freeze drying as a final processing step, which lead to poorly resolved $^{13}$C NMR spectra of little utility. It should be emphasized that most CD complexes exist as three-component systems of host:guest:water. Water appears to play an important role in defining the macrocycle conformation and guest dynamics in CD inclusion complexes (Ripmeester, 1988). Water also plays an important role in determining both long and short range order in the CDs, therefore, it is imperative to prepare samples that are single-phase, uniformly hydrated materials to acquire optimum spectra (Ripmeester and Ratcliffe, 1996). Hydration level significantly affects the multiplicity of resonances and chemical shift dispersion in $^{13}$C CP/MAS spectra of CD and their inclusion complexes (Cunha-Silva and Teixeira-Dias, 2002; Cunha-Silva and Teixeira-Dias, 2004; Cunha-Silva and Teixeira-Dias, 2005). The spectra of small amphiphilic molecules included in β-CD appear as amorphous solids at relative humidities lower than 15%, which indicates a crystalline structure collapse due to the loss of water at low hydration levels (Cunha-Silva and Teixeira-Dias, 2005).

In the solid state, nuclei which are typically considered to be chemically equivalent may not be and unexpected multiplicities may result. All crystallographically inequivalent nuclei in the crystal asymmetric unit should produce separate resonances, although accidental degeneracies are often experienced (Ripmeester and Ratcliffe, 1996). Therefore, the $^{13}$C NMR spectrum can provide information on the content of the smallest repeat unit in the crystal. α-CD and β-CD have been reported to have a single host molecule in the asymmetric unit, which results in spectra with six and seven resonances, respectively, for each carbon in the glucose unit (Gidley and Bociek, 1986). CDs are always distorted from regular geometry in the solid state, and although the molecules have flexibility, there is little evidence for large amplitude motions on an NMR timescale.

Chemical shift data from the $^{13}$C NMR spectrum is limited in providing specific geometrical features. The presence of a wide spread in chemical shift values for the C1 and C4 carbon multiplets generally indicates a large variation in the torsional angle, which suggests a CD geometry noticeably distorted from that in solution (Ripmeester and Ratcliffe, 1991). CDs appear to exist in locked conformations in the solid state based on $^{13}$C NMR and diffraction data. Therefore, the $^{13}$C chemical shifts for CD in solution are close to values obtained by averaging the shifts of individual resonances acquired for the solids (Ripmeester and Ratcliffe, 1991). This
would be the expected result for the presence of many different conformers in a state of rapid exchange.

CD complexation of a guest molecule results in changes in the $^{13}$C CP/MAS spectrum and may indicate conformational effects, in addition to local magnetic environmental effects caused by the guest. While a single resonance is obtained in solution, the multiple resonances observed in solid-state NMR spectra, may be due to the presence of more than one molecule in the asymmetric unit of the crystal structure, the imposition of a crystallographic site symmetry that is less than the overall molecular symmetry, or the removal of conformational averaging in the solid state (Heyes and others 1992). The $\beta$-CD and $\gamma$-CD spectra in the solid state have averaged chemical shifts for the C-1,4,6 resonances that are similar to those in solution while the C-2,3,5 resonances overlap and cannot be assigned individually.

The $^{13}$C CP/MAS NMR spectra of $\alpha$, $\beta$, and $\gamma$-CD have been reported at various hydration levels between fully hydrated, the level which is most stable for each CD at room temperature and humidity, and fully dehydrated (Heyes and others, 1992). The removal of the first water results in the resonance intensities changing and new resonances appearing followed by the resonances broadening as more water is removed. This broadening is presumed to be due to the conformations of the CD torus adapting to changes in the hydrogen-bonding environment, which results in a dispersion in the torsion angles. The resonances become very broad upon removal of nearly all the water, reflecting the more amorphous nature of the CD. The amorphous state observed is expected to be the result of the removal of the intercavity water molecules causing the stacking order of the CD molecules to be severely disrupted.

Several mechanisms can account for the fading out of specific resonances in $^{13}$C CP/MAS NMR spectra. Motional effects can considerably affect the guest portion of the spectrum when using a spectrum for identification of CD complex formation (Ripmeester and Ratcliffe, 1991). Line broadening typically appears in $^{13}$C CP/MAS spectra when molecular motion is present at certain specific frequencies (Ripmeester and Ratcliffe, 1991). The disappearance of the multiple resonances for each carbon type of the CD is a characteristic indication of formation of an inclusion complex (Garbow and others 2001; Porbeni and others 2001; Lai and others 2003; Potrzebowski and Kazmierski, 2004; Anzai and others 2006). Chemical shifts of guest and host CD carbons are often negligibly changed by the complexation, but the resonances experience marked broadening. This broadening has been attributed to the complex existing in an
amorphous state, which includes various complexes with slightly different conformations, inclusion modes, and orientations (Anzai and others, 2006). The multiplicity of carbon resonances and chemical shift dispersion is progressively reduced along a series of nonionic surfactant inclusion complexes of increasing chain length (Cunha-Silva and Teixeira-Dias, 2002). These guest molecules interact progressively with β-CD through proton-proton intermolecular contact interactions due to the alkyllic chains of these guests becoming increasingly more flexible.

**Thermoanalytical Characterization**

The thermal analysis of CD complexes has been used to differentiate between inclusion complexes and adsorbates, and to characterize the unique thermal effects due to molecular entrapment during a distinct, standard heating process. Most of the water molecules within the CD cavity are released at <100 °C in the native CDs. Complexes must have a guest with a melting or boiling point below the thermal degradation range of the CD or must be volatile in the temperature range 60–250 °C to be studied by these methods (Szente, 1996a). Thermal analysis has mainly been applied to demonstrate the different behavior of an inclusion compound relative to its physical mixture of component compounds (von Plessing Rossel and others 2000).

**Synthetic Antioxidant Release from Food Packaging**

Migration typically describes a diffusion process, which may be significantly affected by the interaction of food components with the packaging material (Arvanitoyannis and Bosnea, 2004). Many compounds commonly migrating from packaging materials to foods are polymer additives, monomers, oligomers, and contaminants. Several types of additives are typically incorporated into polymers at concentrations of 0.01–1.0% (w/w) to limit the effects of oxidative degradation during processing and the lifetime of the polymer (Calvert and Billingham, 1979). These additives may include plasticizers, antioxidants, light stabilizers, thermal stabilizers, lubricants, antistatic agents, antifog agents, and slip additives. Any soluble additive may be removed from a polymer by two separate processes: (a) the removal of material from the surface by evaporation or dissolution and (b) its replacement in the surface layer by diffusion from the bulk polymer phase. Calvert and Billingham (1979) studied the rate of loss of a simple low molecular weight compound from thick polymer slabs and from thin films and fibers. They concluded that the rate
loss of low molecular weight compounds from a thick polymer slab was determined by bulk phase diffusion, and the loss from thin films was primarily determined by the surface evaporation rate. Modeling the loss of an antioxidant from a polymer at a specified temperature requires knowledge of its solubility in the polymer, its diffusion coefficient in the polymer, and the volatility of the pure additive (Calvert and Billingham, 1979). The antioxidant solubility in polymer films is described by the solubility coefficient, which is determined by the polymer morphology and the antioxidant’s chemical properties (Shin and Lee, 2003). However, the diffusion of Irganox 1076 was not found to be influenced by polymer morphology in low density polyethylene (LDPE), a 60:40 (w/w) blend of LDPE and linear low density polyethylene (LLDPE), and an ethylene vinyl acetate copolymer with 9% (w/w) vinyl acetate content (Földes, 1993).

Migration of synthetic antioxidants has been a health safety concern and a regulatory problem in many countries. The FDA established a “threshold of regulation” process in the Federal Register on July 17, 1995 (FDA, 1995). The probability of a compound causing a health hazard depends on its dietary concentration and its toxic potency. The FDA considered both of these factors in establishing a threshold of regulation level. Antioxidants and stabilizers are required to be rigorously tested for toxicity before they can be licensed as food contact additives (Scott, 1995). Such additives are frequently degraded during packaging manufacture and use, resulting in secondary migrants of generally unknown and untested toxicity (Scott, 1995). Increased consumption of bottled drinking water increases the risk of greater daily ingestion of antioxidants, such as BHT, due to its migration from the polymer packaging into the water (Tombesi and Freije, 2002).

Antioxidants and stabilizers can be classified into two groups: compounds that prevent formation of free radicals from hydroperoxides and compounds that inhibit the radical chain reaction (Scott, 1995). Figure 2.3 displays the chemical structures of BHT and Irganox 1010, which are the most commonly used antioxidants (Arvanitoyannis and Bosnea, 2004). Antioxidants and stabilizers act sacrificially and are partially or completely converted to secondary products when they function to protect the polymer. The major products formed when BHT is oxidized are electron acceptors and are much more effective than BHT itself as processing stabilizers (Scott, 1995).
Tombesi and Freije (2002) found that BHT was present in 46% of commercial drinking water bottled in polyethylene terephthalate (PET) in Argentina. Schwope and others (1987) proposed a tentative model that explains the behavior of BHT migrating from polymers into aqueous solutions. The first assumption of the model is that BHT initially migrates into water by diffusion from within LDPE to the surface where it dissolves. A partitioning equilibrium is nearly attained as the BHT concentration in the water increases and the migration rate begins to decrease, however, migration is not halted due to the simultaneous decomposition of BHT (Schwope and others, 1987). BHT was believed to have reacted in the aqueous phase and then decomposed to some unknown products. Schwope and others (1987) hypothesized that the BHT concentration in the water reaches a pseudo-steady-state value with continuous migration from the polymer counterbalanced by the decomposition of BHT. Oxidizing agents or impurities in water may also possibly affect the BHT decomposition reaction rate.

The rate of loss of the antioxidant BHA from high density polyethylene (HDPE) was found to follow a first-order expression (Han and others 1987). The mass transfer of BHA was found to be controlled by volatilization rather than diffusion. Greater than 95% of BHA was lost within one day at 50 °C, within three days at 40 °C, and within seven days at 30 °C (Han and others, 1987). Water packaged in LDPE-films may develop off-flavor dependent on extrusion temperature and the content of the oxidation products in the polymer film (Andersson and others 2005a; Andersson and others 2005b). Off-flavor of packaged water is clearly impacted by the presence of aldehydes and ketones, specifically for C7-C9 ketones and C5-C8 aldehydes (Andersson and others, 2005b).

While the migration of synthetic antioxidants into food products is generally of concern, the cereal industry uses the antioxidants BHA or BHT by incorporating these additives into a wax liner (Labuza and Breene, 1989). The antioxidants then diffuse into the cereal flakes during the shelf life of the product to protect the food from lipid oxidation. The kinetics of diffusion of BHT from polyethylene (PE) films was studied in relation to its influence on product stability (Hoojjat and others 1987). About 80% of the BHT diffuses out of the package, but the residual 20% diffuses into the cereal and provides a degree of protection equivalent to when the antioxidant is incorporated directly into the food. Oatmeal cereal packaged in a high-level BHT impregnated HDPE film was observed to have an extended shelf life compared to a low-level BHT impregnated HDPE film (Miltz and others 1988). BHT and BHA-treated PE films in
contact with vegetable oils have been shown to have a stabilizing effect on stored oils against oxidative degradation (Sharma and others 1990). An additional application of BHT-incorporated PE film was the inhibition of lipid oxidation in both fish muscle and oil (Huang and Weng, 1998).

**Natural Antioxidants in Polymers**

The migratory nature of BHT into foods and food simulants has caused some concern regarding its continuous use as an antioxidant in food-packaging materials; therefore, \(\alpha\)-tocopherol has been increasingly tested as a natural alternative. Natural antioxidants such, as \(\alpha\)-tocopherol, are “generally recognized as safe” (GRAS) by FDA and its degradation products containing mostly tocopherol quinone compounds are harmless (Laermer and Zambetti, 1992). \(\alpha\)-Tocopherol would be expected to have a slower migration rate from packaging into a food due to the larger molecular weight of \(\alpha\)-tocopherol (431 g/mol) relative to BHT (220 g/mol). The thermal stability of \(\alpha\)-tocopherol appears far superior to BHT by thermogravimetric analysis showing that \(\alpha\)-tocopherol does not begin to volatilize until almost 300 °C (Laermer and Zambetti, 1992). \(\alpha\)-Tocopherol was observed to be susceptible to thermo-oxidative degradation beginning at 165 °C as measured by non-isothermal DSC measurements (Giuffrida and others 2007).

Lee and others (2004) incorporated \(\alpha\)-tocopherol and BHT into a heat seal layer (Surlyn/EVA®) coextruded with an HDPE layer at initial concentrations of 0.0073 and 0.1137% (w/w), respectively. They determined the first-order rate constants of \(\alpha\)-tocopherol and BHT from the packaging film at 23 °C to be 0.00115 h\(^{-1}\) and 0.0037 h\(^{-1}\), respectively. After 18 weeks of storage at 23 °C, the relative percentage of antioxidant remaining in the packaging film was 36% of \(\alpha\)-tocopherol and only 3% of BHT. After six days of accelerated testing at 45 °C, approximately 25% of \(\alpha\)-tocopherol and no BHT remained in the film (Lee and others, 2004).

Wessling and others (2000a) also found that BHT was depleted much more rapidly than \(\alpha\)-tocopherol during storage at 20, 30, and 40 °C at 50% relative humidity. Importantly, their results indicate that the presence of a food product affects the loss of \(\alpha\)-tocopherol from LDPE film. Oatmeal packaged in these LDPE pouches resulted in a loss of approximately 400 mg/g of \(\alpha\)-tocopherol during four weeks of storage, but the film stored without the product had retained
900 mg/g of $\alpha$-tocopherol during the same period (Wessling and others, 2000a). Different characteristics of the food, such as contact phase properties, fat, alcohol, and acid content, influence the retention of $\alpha$-tocopherol in LDPE films (Wessling and others 1999). $\alpha$-Tocopherol is considered to be a more stable antioxidant than BHT when used in an LDPE film in contact with sunflower oil or 95% ethanol due to the much slower transfer from the polymer film to the fatty food simulants (Wessling and others 1998). Substantial loss of $\alpha$-tocopherol from LDPE in contact with water has been reported but this was attributed to degradation caused by metal ions in the medium (Wessling and others, 1999). Higher retention of $\alpha$-tocopherol was reported when citric acid, which has the ability to form complexes with metal ions, was added to the water in contact with the LDPE film.

In polypropylene (PP) film, $\alpha$-tocopherol retention was found to be nearly complete, however, a significant loss of $\alpha$-tocopherol from LDPE film was observed when the film was in contact with mayonnaise, cream, and low-fat milk (Wessling and others, 1999). This difference in antioxidant release from PP and LDPE may be attributed to the different glass transition temperatures ($T_g$) of the materials. The storage temperature of 4 °C falls within the $T_g$ range for PP (-20 to 10 °C), but is much higher than the $T_g$ for LDPE (approximately -100 °C). The structure of PP would be more ordered and rigid relative to the LDPE structure, which is believed to have reduced the ability of $\alpha$-tocopherol to be released from the polymer (Wessling and others, 1999).

$\alpha$-Tocopherol concentrations of 1000 and 10,000 mg/kg have been used in LDPE films with the dual function of protecting the polymer from oxidative degradation during processing and delaying the onset of oxidation in the packaged food (Wessling and others 2000b). After processing the LDPE films, only about one-third (360 and 3400 mg/kg, respectively) of the $\alpha$-tocopherol originally added to the polymer blend was retained with the remaining lost by manufacture and/or storage. These high levels of $\alpha$-tocopherol showed a positive effect on the oxidation stability of linoleic acid emulsions at low temperatures, but may have detrimental effects on LDPE polymer characteristics (Wessling and others, 2000b). Studies by Al-Malaika and others (1994, 1999) report that the overall tocopherol retention level in PE and PP is generally greater than 80% of the original amount added with high initial concentrations of 1 – 10% (w/w). Unstabilized LDPE loaded with lower initial concentrations of 0.2% (w/w) $\alpha$-
tocopherol leads to considerable loss of parent α-tocopherol during severe processing (Al-Malaika and others, 1994). This extended LDPE processing resulted in approximately 97, 89, 84, and 66% α-tocopherol retention after each of four successive extrusion passes. Siró and others (2006) produced films with retention of about 81% of the initial 2000 mg/kg α-tocopherol concentration in unstabilized LDPE. Heirlings and others (2004) produced stabilized LDPE films with retention of about 84% of the initial 2000 mg/kg α-tocopherol loading.

The color and melt flow stabilizing properties of α-tocopherol in PE and PP are similar and, in some cases, greater than those of Irganox 1010 or 1076 (Ho and others 1994). The high antioxidant activity of α-tocopherol is at least partially the result of the transformation products formed during melt processing (Al-Malaika and others, 1994). The major products formed are diastereoisomers of dimers and trimers in addition to aldehydes, whose relative concentrations are dependent upon processing severity and the initial α-tocopherol concentration. α-Tocopherol has also been shown to be more effective at reducing off-flavors from HDPE at lower concentrations than those required for synthetic antioxidants, such as Irganox 1010 and BHT (Ho and others, 1994). Therefore, the use of α-tocopherol is cost-effective due to these reduced effective concentrations of 250 ppm for PP and 100 ppm for PE (Laermer and Nabholz, 1990; Laermer and Zambetti, 1992). α-Tocopherol exhibits an excellent melt stabilization to PE and PP at very low concentrations in the range of 100–300 ppm, which surpasses that of the stabilization provided by some of the best synthetic hindered phenol antioxidants traditionally used for this purpose (Al-Malaika and others, 1999). Suffield and others (2004) reports the maximum antioxidant effect of mixed tocopherols in PP and PE as 2000 ppm and 700 ppm, respectively.

**Cyclodextrin Interaction with Polymers**

Cyclodextrins in aqueous solution have the ability to extract various nonpolar substances from polymers such as PP (Peiris and others 1999). BHT and an unknown polymer additive or polymer degradation product were extracted from PP in the presence of α-CD, HP-β-CD, and γ-CD solutions. PET films have been prepared by melt processing with 5% (w/w) of free α-CD, which acted as an aldehyde-scavenging agent in aqueous model solutions (Suloff and others 2003). Preliminary results suggest that β-CD within polyvinyl chloride films (PVC) possesses
active inclusion complex forming ability with aroma compounds (Fenyvesi and others 2007). \( \beta \)-CD incorporated into PVC films at 5% (w/w) was found to reduce the migration of phthalate ester into a methanol-water mixture (Sreenivasan, 1996). HDPE incorporated with dispersed free CDs have also been reported to exhibit improved barrier properties of diffusion and transmission rate (Wood, 2001).

**Cyclodextrin Inclusion Compounds in Polymer Matrices**

CDs can be incorporated into polymer films with various additives included within the CD cavity. Fungicide:CD complexes have been incorporated into films used to package hard cheeses and significantly extend the product shelf life by inhibiting rapid mold growth on the cheese surface (Szente and Szejtli, 2004). The antibiotic neomycin sulfate and its \( \beta \)-CD inclusion complex were incorporated into polylactic acid and polycaprolactone films (Huang and others 1999). Several dyes included with \( \alpha \)- and \( \beta \)-CD have been embedded into nylon-6 films and when these films are soaked in hot water, the embedded dye:CD complex is disrupted resulting in the film being dyed from the inside out by migration of the dye (Huang and others 2001b). Embedding small amounts of a flame retardant additive complexed with \( \beta \)-CD directly into PET films by melt processing provides an improved flame retardant product (Huang and others, 2001b; Huang and others 2001a). A CD inclusion complex of a natural antioxidant was incorporated into a food packaging polymer for controlled release for the first time by Siró and others (2006), and remains the only study in the literature to date.

The incorporation of CDs into polymer matrices can modify the mechanisms by which an additive is released. For example, physically mixed and complexed CDs can modify additive solubility or diffusivity, improve hydration of the polymer matrix, or contribute to its erosion (Bibby and others 2000). The addition of CDs can increase additive release from a polymer matrix by: (a) improving the aqueous solubility of the additive, (b) functioning as channeling agents and promoting erosion of the matrix, (c) acting as wicking agents, or (d) increasing the concentration of diffusible species (Bibby and others, 2000). The release of additive from polymer matrices with added CDs can be reduced by (a) increasing its molecular weight during complexation, which results in lowered diffusivity; (b) lowering the concentration of diffusible species by forming poorly soluble complexes; (c) reducing the concentration of diffusible species
by forming complexes in which the host is covalently bound to the polymer; or (d) functioning as cross-linking agents and reducing polymer mesh size (Bibby and others, 2000).

The diffusion of guest molecules is reduced upon their complexation with a CD molecule, since this represents a 3 to 25-fold increase in the molecular weight (Szejtli, 1988). In homogeneous solution, the diffusion rate of complexes is always lower than that of the free guest. However, in the case of guest compounds with poor solubility, the enhancement in solubility overcompensates the decrease of the diffusion rate constant. As a result, the observed diffusion rate in most cases exhibits a considerable increase (Szejtli, 1996b). The presence of β-CD significantly affected the drug delivery of nicardipine from a crosslinked polyethyleneglycol matrix by decreasing its effective diffusivity (Quaglia and others 2001). The higher the ratio of CD to nicardipine, the slower was the release from the polymer matrix.

**Linear Low Density Polyethylene**

Linear low density polyethylene (LLDPE) has experienced the fastest growth rate in usage of the major PE families and now comprises approximately 25% of the annual production of PE worldwide (Simpson and Vaughan, 2003). Conventional LLDPE differs from LDPE by having a narrower molecular weight distribution (MWD) and a lack of long-chain branching. All PE is semicrystalline at densities greater than 0.86 g/cm³. LLDPE is produced by the copolymerization of ethylene and α-olefins, most commonly 1-butene, 1-hexene, 4-methyl-1-pentene, and 1-octene. Single-site catalyzed LLDPE has comonomer much more uniformly distributed along the polymer backbone than do conventional resins. A broad range of structural morphologies exist in ethylene-octene copolymers from highly crystalline lamellae to low crystallinity granular.

Zielger catalysts can generally be defined as PE catalysts derived from transition-metal halides and main group metal alkyls. There are multiple active sites which may only be a fraction of the total metal centers as with most heterogeneous systems. Multiple sites lead to PE chains with varying structures across the polymer chains, with a general polydispersity index in the range from 3.5 to 6. Conventional multi-site Ziegler-Natta (ZN) LLDPE has more comonomer in the lower molecular weight fraction and less in the high molecular weight fraction (Simpson and Vaughan, 2003).
The discovery of metallocene (met) catalysts based on the early transition metals (Zr, Ti, Hf) lead to the emergence of single-site catalysts (Muñoz-Escalona and others 1999). Metallocene catalysts are highly active catalysts exhibiting an exceptional ability to polymerize olefin monomers, and have advantages over the conventional ZN and chromium catalysts. Metallocenes produce extremely uniform polymers and copolymers of narrow MWD and narrow comonomer distribution (CMD), and simultaneously control the resulting polymer chain architectures. The first commercial met LLDPE was introduced to the market for applications such as food packaging and impact modifiers and are available with resin densities ranging from 0.86 to 0.93 g/cm$^3$ (Lue, 1999). These products have beneficial properties of outstanding heat seal performance, high gas permeability, excellent clarity, and superior toughness. The first commodity met PEs were introduced to the market to compete with ZN LLDPE’s for applications such as can liners and stretch films. New met PE products are focusing on the control and manipulation of MWD, CMD, long-chain branch, and long-chain branch distribution. Future met PE development will involve manipulating these molecular structures to meet a specific set of performance requirements.

**Controlled Release of Active Ingredients in Foods**

Controlled release is a novel technology which began in the biomedical field and has spread to other research areas including the food industry. Controlled release may be defined as a process by which one or more active ingredients are made available at a desired site and time at a specific rate. Controlled release offers the following advantages: (a) active ingredients are released at controlled rates over prolonged periods of time, (b) ingredient loss during mechanical or thermal processing can be avoided or limited due to increased stability, and (c) reactive or incompatible components can be separated (Pothakamury and Barbosa-Cánovas, 1995).

In the food industry, the most commonly applied method to attain controlled release is microencapsulation. Microencapsulation is defined as the technology of packaging solid, liquid, or gaseous materials in minute sealed capsules that release their contents at controlled rates under the influence of specific conditions. CDs can be considered as empty capsules of molecular size that form complexes with guest molecules resulting in an encapsulation process on the molecular scale. Molecular encapsulation is unique compared to traditional encapsulation because an
effective protection is available for every single guest molecule present in a multicomponent food system (Szente and Szejtli, 2004).

The majority of release profiles by a controlled release system can be categorized into the following types: (a) zero-order release, (b) first-order release, and (c) square-root-of-time (Baker, 1987). **Zero-order release** is the simplest profile where the release rate remains constant until the device no longer contains an active agent. The release rate \( \frac{dM_t}{dt} \) from such a device is given as

\[
\frac{dM_t}{dt} = k,
\]

where \( k \) is a constant, time is \( t \), and the mass of active agent released is \( M_t \).

During **first-order release** kinetics, the release rate is proportional to the mass of active agent contained within the device

\[
\frac{dM_t}{dt} = k(M_0 - M_t),
\]

where \( M_0 \) is the mass of agent in the device at \( t = 0 \). After integration and rearrangement, this gives

\[
\frac{dM_t}{dt} = kM_0 \exp(-kt).
\]

The rate declines exponentially with time in first-order release, approaching a release rate of zero as the device approaches emptiness.

The **square-root-of-time** or \( t^{1/2} \) release displays compound release that is linear with the reciprocal of the square root of time. The release rate is given as

\[
\frac{dM_t}{dt} = \frac{k}{\sqrt{t}}.
\]

The release rate of the square-root-of-time release remains finite as the device approaches the empty state. The release profiles for each of the major types of devices are depicted in Figure 2.4.

**Diffusion in Polymers**

Diffusion is the process by which an additive is transported from a polymer system to a food simulant system as a result of random molecular motions. Migration is the sum of both diffusion and partitioning. The diffusion coefficient \( (D) \) represents the additive migration rate and the
partition coefficient \((K)\) represents the ratio of the additive concentration in the packaging to the additive concentration in the food simulant at equilibrium. The mathematical theory of diffusion in isotropic substances through unit area of a section is proportional to the concentration gradient measured normal to the section, represented as

\[
F = -D \frac{\partial C}{\partial x}
\]  

(9)

where \(F\) is the rate of transfer per unit area of section; \(C\) is the concentration of diffusing substance; \(x\) is the space coordinate measured normal to the section; and \(D\) is called the diffusion coefficient. The fundamental differential equation of diffusion in an isotropic medium is derived from equation 9 and may be reduced to equation 10 if diffusion is one-dimensional.

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}
\]

(10)

The European Thematic Network collected numerous data-sets of experimental \(D\) values in polyolefins at 23 °C (Helmroth and others 2002a). Figure 2.5 compares different models for predicting the \(D\) as a function of migrant molecular weight with experimental data for LLDPE and LDPE. The amount of polymer additive that may migrate into a food or food simulant solvent is dependent upon numerous factors including the original concentration of the additive in the polymer, its solubility in the solvent, its partition coefficient between the polymer and solvent, temperature, and time (Chang and others 1988).

The diffusion rate of isooctane was found to be significantly higher than that of ethanol in LDPE, which implies that isooctane migration enhanced the migration rate of the other components (Piergiovanni and others 1999). The low absorption (<2%, v/v) of the triglycerides had no effect on the migration rate of Irganox 1076 from thin LDPE film (Helmroth and others 2002b). The migration behavior of this synthetic antioxidant from LDPE film to a triglyceride was only dependent on its own migration rate in LDPE and its solubility in the triglyceride.

**Diffusion in a Plane Sheet**

**Diffusion from a Stirred Solution of Limited Volume**

The mathematical solution for a normal Fickian diffusion process with fixed boundary conditions, as the additive migrates from a plane sheet into a stirred liquid, has been solved by Crank (1975). The numerical evaluation of the solution generally is dependent upon series expansions and converges very slowly for early times or small amounts of additive migrating.
The most rigorous general model for describing the migration controlled by Fickian diffusion in a packaging film is given by (Chung and others 2002):

\[
\frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp \left[ -\frac{Dq_n^2 t}{L_p^2} \right]
\]

(11)

where \(M_{F,t}\) is the mass of additive in the food at time \(t\); \(M_{F,\infty}\) is the mass of additive in the food at infinite time; \(\alpha\) is the mass ratio of additive in the food to that in the polymer film at equilibrium; \(q_n\) are the non-zero positive roots of \(\tan q_n = -\alpha q_n\); \(D\) is the diffusion coefficient; \(L_p\) is the half thickness of the polymer film; \(t\) is time; and \(n\) is the index variable. The concentration mass ratio, \(\alpha\), was calculated using:

\[
\alpha = \frac{K_{FP}V_F}{V_P}
\]

(12)

where \(K_{FP}\) is the partition coefficient for the distribution of additive between food, \(F\), and polymer, \(P\); \(V_F\) is the volume of the food; and \(V_P\) is the volume of the polymer.
### Table 2.1. Physical and Chemical Properties of α-, β-, and γ-Cyclodextrin (Szejtli, 1998)

<table>
<thead>
<tr>
<th>Property</th>
<th>α</th>
<th>β</th>
<th>γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of glucose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>molecular weight</td>
<td>972</td>
<td>1135</td>
<td>1297</td>
</tr>
<tr>
<td>water solubility, g/100 mL at room temp.</td>
<td>14.5</td>
<td>1.85</td>
<td>23.2</td>
</tr>
<tr>
<td>cavity diameter, Å</td>
<td>4.7 - 5.3</td>
<td>6.0 - 6.5</td>
<td>7.5 - 8.3</td>
</tr>
<tr>
<td>height of torus, Å</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>outer diameter, Å</td>
<td>14.6 ± 0.4</td>
<td>15.4 ± 0.4</td>
<td>17.5 ± 0.4</td>
</tr>
<tr>
<td>approx. cavity volume, Å³</td>
<td>174</td>
<td>262</td>
<td>427</td>
</tr>
<tr>
<td>approx. cavity volume in 1 mol CD, mL</td>
<td>104</td>
<td>157</td>
<td>256</td>
</tr>
<tr>
<td>crystal water, wt. %</td>
<td>10.2</td>
<td>13.2 - 14.5</td>
<td>8.13 - 17.7</td>
</tr>
<tr>
<td>pK (by potentiometry) at 25 °C</td>
<td>12.332</td>
<td>12.202</td>
<td>12.081</td>
</tr>
<tr>
<td>crystal forms (from water)</td>
<td>hexagonal</td>
<td>monoclinic</td>
<td>quadratic</td>
</tr>
<tr>
<td></td>
<td>plates</td>
<td>parallelograms</td>
<td>prisms</td>
</tr>
</tbody>
</table>
FIGURES

\(\alpha\)-Tocopherol

Anthocyanins

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁, R₂, R₃, R₄, R₅, R₆</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>callistephin</td>
<td>R₁, R₂ = H; R₃ = O-glucose</td>
<td></td>
</tr>
<tr>
<td>chrysanthemin</td>
<td>R₁ = OH; R₂ = H; R₃ = O-glucose</td>
<td></td>
</tr>
<tr>
<td>malvidin</td>
<td>R₁, R₂ = OCH₃; R₃ = OH</td>
<td></td>
</tr>
</tbody>
</table>

Flavonols

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁, R₂, R₃, R₄, R₅, R₆</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-hydroxyflavone</td>
<td>R₁, R₂, R₃, R₄, R₅ = H; R₆ = OH</td>
<td></td>
</tr>
<tr>
<td>morin</td>
<td>R₁, R₂, R₃, R₅, R₆ = OH; R₄ = H</td>
<td></td>
</tr>
<tr>
<td>quercetin</td>
<td>R₁, R₂, R₃, R₅, R₆ = OH; R₄ = H</td>
<td></td>
</tr>
<tr>
<td>rutin</td>
<td>R₁, R₂, R₃, R₅ = OH; R₄ = H; R₆ = O-glucose-rhamnose</td>
<td></td>
</tr>
</tbody>
</table>

Flavonones

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁, R₂, R₃</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>hesperetin</td>
<td>R₁, R₂ = OH; R₃ = OCH₃</td>
<td></td>
</tr>
<tr>
<td>hesperidin</td>
<td>R₁ = O-glucose-rhamnose; R₂ = OCH₃</td>
<td></td>
</tr>
<tr>
<td>naringenin</td>
<td>R₁, R₂ = OH; R₃ = H</td>
<td></td>
</tr>
<tr>
<td>naringin</td>
<td>R₁ = O-glucose-rhamnose; R₂ = H; R₃ = OH</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.1. Structures of various natural antioxidants.
Figure 2.2. (a) The truncated cone shape and the functional structural scheme of the β-cyclodextrin molecule. (b) The glucose units are connected through glycosidic α-1,4 bonds. The C-2-OH group of one glucose unit forms an intramolecular hydrogen bond with the C-3-OH group of the adjacent glucose unit.
Figure 2.3. Synthetic antioxidants used in polymers for food packaging.
Figure 2.4. (a) Zero-order, (b) first-order, and (c) $t^{1/2}$ release patterns from devices containing the same initial active agent content.
Figure 2.5. Comparison of different models for predicting the diffusion coefficient as a function of migrant molecular weight with experimental data for LLDPE and LDPE (Helmroth and others, 2002a). Experimental data (×), deterministic approach (——), worst-case approach (----), and stochastic approach (-----) with approximate 95% confidence interval (------).
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CHAPTER 3: FORMATION AND CHARACTERIZATION OF CYCLODEXTRIN INCLUSION COMPLEXES OF α-TOCOPHEROL AND QUERCETIN

ABSTRACT

Cyclodextrin (CD) complexation procedures are relatively simple processes, but these techniques often require very specific conditions for each individual guest molecule. Variations of the coprecipitation from aqueous solution technique were optimized for the CD complexation of the natural antioxidants, α-tocopherol and quercetin. Solid inclusion complex products of α-tocopherol:β-CD and quercetin:γ-CD had molar ratios of 1.7:1, which were equivalent to 18.1% (w/w) α-tocopherol and 13.0% (w/w) quercetin. The molar reactant ratios of CD:antioxidant were optimized at 8:1 to improve the yield of complexation. The product yields of α-tocopherol:β-CD and quercetin:γ-CD complexes from their individual reactants were calculated as 24 and 21% (w/w), respectively. ATR/FT-IR, $^{13}$C CP/MAS NMR, TGA, and DSC provided evidence of antioxidant interaction with CD at the molecular level which indicated true CD inclusion complexation in the solid state. Natural antioxidant:CD inclusion complexes may serve as novel additives in controlled-release active packaging to extend the oxidative stability of foods.

KEYWORDS
cyclodextrin, inclusion complex, α-tocopherol, quercetin, natural antioxidant, coprecipitation, complexation
INTRODUCTION

The preparation of cyclodextrin (CD) inclusion complexes is often a relatively simple procedure; however, in most cases the reaction conditions have to be customized for the specific guest molecule. The majority of CD complexation reactions occur in aqueous solution or at least in the presence of water. The natural antioxidants, α-tocopherol and quercetin (Figure 3.1), were selected as the specific guest molecules in this study. Tocopherols are the most important natural antioxidants in vegetable oils and protect these products from lipid peroxidation. Quercetin, found in onions and apples, is among the most effective of the flavonoid antioxidants and the most active in its own flavonol class (1). The complexation procedures of α-tocopherol and quercetin with the native CDs that are described in literature are deficient. These specific complexation methods proposed for each of the natural antioxidants require careful review to ensure that true inclusion complexes are indeed being formed.

Solid β-CD:α-tocopherol complexes have been prepared using kneading (2), co-evaporation (2), and freeze-drying methods (2, 3). Both the kneading and co-evaporation methods used 1:1 and 2:1 molar ratios of β-CD:α-tocopherol mixed with different respective volumes of 50% aqueous methanol. Organic solvents usually decrease the association constants of CD complexes relative to pure water. The most commonly proposed idea for this behavior is that increasing the organic content of the aqueous mixture decreases the hydrophobic driving force, which is a major contributor to the stability of the complex in water (4). Methanol and ethanol are often used as cosolvents to aid the solubilization of very hydrophobic guest molecules, but not at the high percentages of methanol reported. CD complexes are typically dissolved in 50% aqueous ethanol followed by dilution with pure ethanol as part of the dissociation procedure for guest content determination (5).

Inclusion complexes of quercetin with β-CD have been prepared by a freeze-drying method from aqueous solution with equimolar ratios of components under strongly alkaline pH conditions (6, 7). The use of ammonia to dissolve quercetin raises the pH to alkaline levels which allows for the formation of the ionized species of quercetin. The ionized quercetin is susceptible to degradation at pH levels greater than 5 (8, 9). This quercetin anion also exhibits much weaker binding with two β-CD derivatives compared to the unionized species due to its hydrophilic character (8). Preparation of solid inclusion complexes of quercetin with α- and β-CD has also been reported by kneading, co-evaporation, and thin layer methods (10).
products were obtained by wetting equimolar physical mixtures with a minimum volume of 50% aqueous methanol. Co-evaporated products were obtained from 33.3% methanol in aqueous solution containing equimolar amounts of quercetin and CD. Guest–host associations are prevented with the addition of a high percentage of alcohol for the short chain alcohols, such as methanol and ethanol (11). Longer chain alcohols, such as pentanol or even greater length, are able to cancel this association with only 1% alcohol. Similar to the α-tocopherol methods reported in literature, the use of relatively high percentages of methanol cosolvent may result in either very weak associations or no complexation at all.

Several researchers have also reported solubility increases of quercetin during phase solubility studies with β-CD from its intrinsic water solubility of 0.44 mg/L (8) to approximately 2–25 mg/L (6, 8, 12). Although, an increase in the water solubility of the flavonols has been reported in the case of physical mixtures and some kneaded preparations when no inclusion complexes were in fact formed (13). The limited solubility increase of quercetin in the presence of β-CD, therefore, only provides weak evidence of an association, while quercetin still remains practically insoluble.

The purpose of this research was to optimize the complexation methods of the natural antioxidants, α-tocopherol and quercetin, and characterize their cyclodextrin inclusion complexes. Solid-state techniques, including ATR/FT-IR, 13C CP/MAS NMR, TGA, and DSC, provided a clear indication of guest interactions with their CD hosts at the molecular level. Molecular encapsulation with CDs can function to protect against oxidation and heat-induced degradation of guest molecules. CD complexation can provide oxidative protection to flavors (vanillin (14), thymol, and geraniol (15)) and antioxidants (ferulic acid (16)). The oxidative sensitivity of α-tocopherol (17-20) and quercetin (9) has been observed during extended thermal processes. CD inclusion complexes of the natural antioxidants were formed for their potential thermal and oxidative stability and controlled-release properties for the future incorporation of these antioxidants into active food packaging.

MATERIALS AND METHODS

Materials. (±)-α-Tocopherol of 98% purity and quercetin dihydrate of 99% purity were supplied by Sigma-Aldrich (St. Louis, MO). Food-grade α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) were kindly donated by Wacker Fine Chemicals (Adrian, MI).
Potassium phosphate monobasic (KH$_2$PO$_4$), methanol, and water of HPLC grade, and 99.5% ethanol of ACS reagent grade were obtained from Fisher Scientific (Pittsburgh, PA).

**Preparation of α-Tocopherol:β-CD Complex.** Solid inclusion complexes of α-tocopherol with β-CD were obtained by coprecipitation from aqueous solution. A 16.0 mM β-CD aqueous solution was prepared followed by addition of 2.0 mM α-tocopherol. These concentrations were chosen for their relatively high complexation yield based on preliminary studies. The dispersion of α-tocopherol in the aqueous β-CD solution was protected from light and mechanically shaken at 25 °C and 250 rpm in an Innova 4230 refrigerated incubator shaker (New Brunswick Scientific, Edison, NJ) for 24 hours to achieve equilibrium of the complexation reaction. Samples were allowed to settle gravimetrically and the majority of supernatant was decanted. The remaining coprecipitate was collected by filtering the remaining supernatant through a 0.2-μm nylon membrane. These solid complexes were frozen at -20 °C and then lyophilized in a laboratory freeze-dryer (Virtis, Gardiner, NY). CD complexes of α-tocopherol were dissociated in 99.5% ethanol for a period of 24 hours at 25 °C and 250 rpm. Guest content of each CD complex was quantified by UV spectrophotometry. Solid α-tocopherol:β-CD complexes were produced in triplicate and complexes with 18.1% (w/w) α-tocopherol content were used for all characterization tests.

**Preparation of Quercetin:γ-CD Complex.** Solid inclusion complexes of quercetin with γ-CD were obtained by coprecipitation from aqueous solution with 10% ethanol as cosolvent. A solution of 26.7 mM γ-CD was prepared in 0.05 M potassium phosphate buffer at pH 3.0 (8). A 30.0 mM quercetin dihydrate solution in ethanol was prepared and added to the γ-CD solution to give a final concentration of 24.0 mM γ-CD and 3.0 mM quercetin in an aqueous solution with 10% ethanol. This final solution of γ-CD and quercetin was protected from light and mechanically shaken at 25 °C and 250 rpm in an incubator shaker for 24 hours to achieve equilibrium of the complexation reaction. Samples were allowed to settle gravimetrically and the majority of supernatant was decanted. The coprecipitate was collected by filtering the remaining supernatant through a 0.2-μm cellulose nitrate membrane. Quercetin solutions were observed to adsorb to nylon membranes. These solid complexes were frozen at -20 °C and then
lyophilized in a laboratory freeze-dryer. CD complexes of quercetin were dissociated in 50% aqueous ethanol for a period of 24 hours at 25 °C and 250 rpm. Guest content of each CD complex was quantified by UV spectrophotometry. Solid quercetin:γ-CD complexes were produced in triplicate and complexes with 13.0% (w/w) quercetin content were used for all characterization tests.

Preparation of Physical Mixtures. Physical mixtures of α-tocopherol and β-CD were prepared with the identical α-tocopherol content of 18.1% (w/w) as in its solid CD inclusion complex by thoroughly mixing the two components. Physical mixtures of quercetin dihydrate and γ-CD were prepared with the identical quercetin dihydrate content of 13.0% (w/w) as in its solid CD inclusion complex in the same manner.

UV Absorption Spectrophotometry. Spectrophotometry was performed with a UV-2101PC UV-VIS scanning spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) to quantify α-tocopherol and quercetin in their free and CD complexed forms (n = 2). Standard curves of α-tocopherol and quercetin were prepared in 99.5% ethanol, accounting for the sample purity of each natural antioxidant. CD complexes of α-tocopherol and quercetin were dissociated in 99.5% ethanol and 50% aqueous ethanol, respectively, for 24 hours at 25 °C. Complete spectrophotometric scans between 250 and 400 nm were performed to monitor any changes in the UV spectra of the natural antioxidants. The absorbance maxima of α-tocopherol and quercetin were 292 and 375 nm, respectively, to quantify each antioxidant concentration.

Colorimetric Analysis. A Chroma Meter CR-200 tristimulus color analyzer (Minolta, Ramsey, NJ) was calibrated with a standard white plate (L 97.29, a -0.18, b +3.75) and used for measuring reflective colors of surfaces. Solid CD inclusion complexes, physical mixtures, guest antioxidants, and CDs were measured with the color analyzer and quantified using the CIE L*a*b* color model. ΔE*ab was used to quantify the magnitude of the total color difference as a single numerical value defined by the equation: ΔE*ab = [(ΔL*)2 + (Δa*)2 + (Δb*)2]1/2.
**CD Sample Equilibration at Ambient Relative Humidity.** Uncomplexed CD, physical mixtures, and inclusion complexes of 1.0 g sample weight in 20 mL glass vials were left open in a sealed glass desiccator with distilled water filled across the base of the desiccator for an equilibration period of 120 min. This 100% relative humidity environment completely hydrated the CD-containing samples through the vapor phase on the minute time scale as reported in literature (21, 22) and confirmed in a preliminary study (Figure 3.7). The guest molecules, \( \alpha \)-tocopherol and quercetin dihydrate, were not placed in the 100% relative humidity environment. The same CD-containing samples were then left open to ambient relative humidity for an equilibration period of 120 min. This hydration and dehydration step was to allow the CD inclusion complexes of both \( \alpha \)-tocopherol and quercetin to be at equivalent hydration states as the uncomplexed CD and physical mixtures since during their production the complexes were lyophilized. Characterization techniques of ATR/FT-IR, \(^{13}\)C CP/MAS NMR, TGA, and DSC were performed on these samples equilibrated at ambient relative humidity.

**ATR/FT-IR Spectroscopy.** Attenuated total reflectance/Fourier transform-infrared (ATR/FT-IR) spectroscopy was performed on a Spectrum One FT-IR spectrometer (Perkin Elmer, Waltham, MA). A Miracle single reflection ATR sampling accessory (PIKE Technologies, Madison, WI) was used with a ZnSe crystal plate and a micrometric, low-pressure clamp. Background scans were recorded between 4000 to 600 cm\(^{-1}\) with 164 scans. Samples were scanned 64 times at a resolution of 4.00 cm\(^{-1}\). Absorbance spectra were processed by the ATR correction feature of the Spectrum v.5.0.1 (Perkin Elmer, Waltham, MA) software package with a contact factor of 0 to correct for the variation in effective pathlength.

**\(^{13}\)C CP/MAS NMR Spectroscopy.** \(^{13}\)C cross polarization/magic angle spinning (CP/MAS) NMR of hydrated sample powders was performed on a Bruker Avance II 300 operating at 75.48 MHz for \(^{13}\)C and equipped with a MAS probehead using 4-mm ZrO\(_2\) rotors. Chemical shifts were calibrated with an external standard of 1-glycine at 176.4 ppm. Samples were spun at 5 kHz at room temperature. Spectra were acquired with a proton 90° pulse length of 5 \(\mu\)s and a \(^{13}\)C-\(^1\)H contact time of 2 ms. The repetition delay time was 2 s and the spectral width was 25 kHz. Free induction decays were accumulated with a time domain size of 1K data points. A square-shaped pulse was used during the cross-polarization and a TPPM decoupling pulse.
sequence with a phase angle of 15° was used during the acquisition. Each sample spectrum was obtained with 2048 scans and processed with 20-Hz line broadening. $^{13}$C NMR of $\alpha$-tocopherol was performed using a single 90° pulse for 5 $\mu$s without CP/MAS since it exists as an oily liquid at room temperature. $^{13}$C resonances were assigned in spectra based on reported data of $d$-$\alpha$-tocopherol (23), quercetin (24, 25), and $\beta$-CD and $\gamma$-CD (26).

**Thermogravimetric Analysis.** A TGA Q500 thermobalance (TA Instruments, New Castle, DE) was used to measure sample weight loss to determine water content and thermal decomposition temperature. The thermobalance was calibrated with an alumel alloy and nickel for temperature settings and with a 100-mg standard for weight accuracy. Sample (5.5 ± 0.5 mg) was placed on a tared aluminum balance pan and transferred to the furnace at room temperature, where the exact sample weight was determined. The temperature program increased the temperature at a rate of 5 °C/min from 30 to 500 °C under an air atmosphere. Universal Analysis 2000 (TA Instruments, New Castle, DE) software was used to determine decomposition temperatures using the maximum of the derivative thermogravimetric curve.

**Differential Scanning Calorimetry. Enthalpies of Dehydration and Vaporization.** A DSC Q1000 instrument (TA Instruments, New Castle, DE) was used to determine the dehydration and vaporization enthalpies of samples. A standard temperature ramp was used from equilibration at 0 °C followed by an increase to 250 °C at a rate of 5 °C/min. A N$_2$ sample purge flow was used at 50 mL/min. A hermetic aluminum sample pan and lid with a laser-drilled 75-$\mu$m pinhole (TA Instruments, New Castle, DE) was used to hold samples with weights of 2.3 ± 0.2 mg. TA Universal Analysis 2000 software was used to process data.

$T_g$ of $\alpha$-Tocopherol. The glass transition temperature ($T_g$) of $\alpha$-tocopherol, $\alpha$-tocopherol and $\beta$-CD physical mixture, and $\alpha$-tocopherol:$\beta$-CD inclusion complex was determined by DSC. A standard temperature ramp was used from equilibration at -90 °C followed by an increase to 0 °C at a rate of 5 °C/min. A N$_2$ sample purge flow was used at 50 mL/min. A hermetic aluminum sample pan and lid without pinhole was used to hold samples with weights of 5.1 mg. TA Universal Analysis 2000 software was used to perform $T_g$ analysis and calculate the midpoint $T_g$. 
RESULTS AND DISCUSSION

Preparation of Solid CD Inclusion Complexes. Preliminary Studies. Various different complexation techniques were explored for the natural antioxidants with the native CDs. The complexation in aqueous solution technique (27) used previously was not effective for 16 mM α-CD, β-CD, or γ-CD in the preparation of water-soluble inclusion complexes with excess (2 mg/mL) α-tocopherol or quercetin dihydrate. Preparation of CD complexes by the co-evaporation method with methanol as cosolvent (33.3%, v/v) in aqueous solution using 2:1 molar ratio of α-CD, β-CD, or γ-CD to guest antioxidant was not successful as had been previously reported with equimolar host:guest ratios (10). The association constant of complexes decreases with a corresponding increase in the hydrophobicity of the medium, which may be due to increasing the percentage of a certain alcohol or increasing the chain length of a certain alcohol (11). The decrease in association constants is steeper as the number of carbons in the alcohol chain increases. Addition of methanol to aqueous solutions of guest and β-CD results in a nonlinear decrease of association constants that has a greater slope at lower and less slope at higher methanol concentrations of 20% (v/v) (28).

Neutral compounds generally have larger complex stability constants than the corresponding protonated or ionized species (29). The pKₐ of α-tocopherol was reported as ≥11.0 (30) while quercetin had pKₐ1 at 7.0 and pKₐ2 at 9.2 (31). α-Tocopherol exists predominantly in the unionized form at neutral pH; therefore, unbuffered water was used as the complexation medium. The presence of quercetin in its ionized form at neutral pH may hinder complexation; therefore, quercetin was complexed in 0.05 M potassium phosphate buffer at pH 3.0.

α-Tocopherol. An equilibration time study of α-tocopherol behavior in aqueous solutions of β-CD was performed over a 168-hour period. At 24 hours, increasing the β-CD:α-tocopherol ratio from 1:1, 2:1, 4:1, 8:1, to 16:1 resulted in complex precipitates of similar yield and α-tocopherol content. Generally a true inclusion complex would be expected to have a molar ratio of CD:guest of greater than one, or at least one host CD site for each guest molecule. However, visual observation clearly evidences that 16 mM α-tocopherol and 16 mM β-CD interacted to form an insoluble precipitate with the CD:guest ratio of the product dropping rapidly by 120 hours to a ratio of about 0.8 at 168 hours (Figure 3.8b). α-Tocopherol is hypothesized to have
interacted with the β-CD molecule to form a higher order complex or other association in a sequential process, but no structural experiments were conducted to prove this hypothesis.

*Quercetin.* After an equilibration time of 24 hours, concentrations of γ-CD were increased in the presence of 3 mM quercetin in 0.05 M potassium phosphate buffer at pH 3 with 10% ethanol (Figure 3.9). CD:quercetin reactant ratios of 1:1 and 2:1 were observed as free quercetin without CD complexation, which was evidenced by CD:quercetin product ratios of less than one. CD:quercetin reactant ratios of 4:1, 8:1, and 16:1 appeared as an inclusion complex as indicated by a precipitate of much lighter yellow intensity and greater density than free quercetin. The CD:quercetin product ratios of these precipitates were additionally greater than one.

Solution complexation techniques in most cases require a water-miscible cosolvent that efficiently dissolves the guest. The direct addition of 3.0 mM quercetin as a solid to a 38.6 mM (5%, w/w) γ-CD aqueous solution without cosolvent resulted in a CD:guest product ratio of about 0.3 with no indication of association observed. Very hydrophobic guests, such as quercetin, may also require a cosolvent. Methanol and ethanol were both evaluated, but quercetin was observed to have a greater solubility in ethanol. The addition of 10% (v/v) ethanol as a cosolvent to the system above resulted in CD:guest product ratios of about 1.7 and a precipitate of lighter yellow intensity, which indicated CD complex formation. β-CD complexation efficiency in aqueous solution has been reported to increase with increasing concentrations of ethanol up to a maximum, beyond which the efficiency decreases (32). The presence of ethanol changes the solvophobic characteristics of the medium, which may affect the affinity of a nonpolar guest in binding CD (33). Ethanol is a commonly applied cosolvent in the preparation of CD complexes, and as a result inclusion products may contain about 0.01–0.5% ethanol which cannot be removed without potentially disrupting the formed CD complexes (34). Ethanol may play a space regulating role in CD complexes by facilitating the formation of a stronger complex by filing the void inside the CD cavity (35, 36).

*Coprecipitation Methods.* A large molar excess of host CD is required to initiate the inclusion complexation reaction of some hydrophobic guests, such as quercetin. An excess of one component is necessary to drive the equilibrium since the concentration of reactants is low and the association constant of CD inclusion complexes is also low (37-39). The molar ratios of CD:antioxidant were optimized at 8:1 to improve the yield of complexation. Table 3.1 lists the ratios of reactants used in complex preparation and the antioxidant content of complexes in terms
of weight percent and CD:antioxidant molar ratio. The stoichiometries of these complexes were unable to be determined. The calculated CD:antioxidant complex molar ratios were not integers, so it may be assumed that a 1:1 complex was present as a mixture with either excess, uncomplexed CD or a higher order 2:1 complex. The product yields of α-tocopherol:β-CD and quercetin:γ-CD complexes from their individual reactants were calculated as 24 and 21% (w/w), respectively. Nearly complete reaction of each natural antioxidant is observed, and the majority of yield losses are due to uncomplexed CD remaining in aqueous solution. Uncomplexed CD could easily be collected by lyophilization or reused directly in additional complexation reactions as part of a continuous production process.

Properties of Solid CD Inclusion Complexes. The guest α-tocopherol exists as an oily liquid at room temperature and this oily character is retained upon physical mixing with β-CD. The formation of its β-CD inclusion complex results in a solid, dry powder. The more solid character of α-tocopherol within the β-CD cavity was later confirmed by 13C CP/MAS NMR spectroscopy.

Solid CD inclusion complexes of natural antioxidants assume more of the physical appearance of their host CD compared to their physical mixtures (Figures 10-11). In Table 3.2, the magnitude of the total color difference ($\Delta E^{*}_{ab}$) from the host CD is shown for each antioxidant, physical mixture, and inclusion complex. The α-tocopherol:β-CD complex is a white powder that is almost indistinguishable from its host β-CD giving it a $\Delta E^{*}_{ab}$ of 1.6. The physical mixture of α-tocopherol and β-CD retains more of the color of α-tocopherol with a $\Delta E^{*}_{ab}$ of 24.2. The quercetin:γ-CD complex is a light yellow powder with a $\Delta E^{*}_{ab}$ of 18.8, while its physical mixture retains more of the bright yellow color of quercetin with a $\Delta E^{*}_{ab}$ of 36.4. These observations of decoloration agree with the expectation that when a colored guest molecule is included within the CD cavity, the inclusion complex produced has lower color intensity (40-42). In this manner, colored guest molecules often provide the benefit of allowing visual observation as the first evidence of inclusion complex formation.

CDs form practically water-insoluble complexes with very hydrophobic guests, such as α-tocopherol and quercetin. The α-tocopherol:β-CD complex was unexpectedly observed to dissociate in water as the β-CD host appears to resolubilize and α-tocopherol accumulates at the
water surface as a dispersion. Quercetin:γ-CD complexes were not observed to exhibit this unique behavior of water dissociation. Investigations of these natural antioxidant:CD complexes are complicated by their lack of water solubility and their dissociation in most organic solvents, which restricts inclusion complex characterization to solid-state techniques.

**ATR/FT-IR Spectroscopy.** The characteristic absorption bands of both natural antioxidants are in the spectral region where CD absorption is limited, which allows the detection of guest interactions within solid CD inclusion complexes. In Figure 3.2, the α-tocopherol spectrum shows intense bands at 2924 and 2867 cm\(^{-1}\) for asymmetrical methylene and symmetrical methyl stretching vibrations, respectively (43, 44). These two intense bands are clearly present in the physical mixture of α-tocopherol and β-CD; however, these bands are no longer apparent upon complexation within β-CD. Characteristic absorption bands of α-tocopherol at 1460 cm\(^{-1}\) for phenyl skeletal and the overlap of asymmetrical methyl bending and methylene scissoring vibration, and 1377 cm\(^{-1}\) for symmetrical methyl bending (43, 44) are similarly not present in the β-CD complex. It is proposed that a tight fitting of α-tocopherol within the β-CD cavity would hinder these molecular vibrations, consequently diminishing the intensities of their absorption bands. The spectrum of the α-tocopherol:β-CD complex appears very similar to that of its β-CD host.

In Figure 3.3, the spectrum of the quercetin:γ-CD complex shows that the band intensity within the 1700- to 1200-cm\(^{-1}\) range, where stretching modes are observed, is suppressed compared to its physical mixture. The carbonyl absorption band of quercetin and its physical mixture is observed at 1665 cm\(^{-1}\) in agreement with literature (45, 46). In the quercetin:γ-CD complex, this carbonyl band of quercetin is shifted 10 cm\(^{-1}\) to the longer wavelength of 1655 cm\(^{-1}\), which is an indication of further hydrogen bonding (47-49). Quercetin is involved in intramolecular hydrogen bonding of its carbonyl oxygen at C4 with the two hydroxyl groups at C3 and C5 (45). In the inclusion complex, the observed shift to 1655 cm\(^{-1}\) suggests that intermolecular hydrogen bonding occurs between the quercetin carbonyl and the γ-CD hydroxyls. The spectrum of the quercetin:γ-CD complex also appears very similar to that of its γ-CD host.
**$^{13}$C CP/MAS NMR Spectroscopy.** The $^{13}$C CP/MAS NMR spectrum of free $\beta$-CD in Figure 3.4 shows splitting of several of the carbon resonances which indicates a rigid, non-symmetric conformation of the CD crystals. The spectrum of the inclusion complex shows all of the major resonances of $\alpha$-tocopherol and $\beta$-CD in Figure 3.4. With the presence of $\alpha$-tocopherol in the $\beta$-CD cavity of the crystal structure, the resonances of the $\beta$-CDs show reduced splitting and broadening which indicates the crystals have adopted more symmetric and possibly dynamic conformations. The simple observation of resonances due to $\alpha$-tocopherol in the inclusion complex provides additional evidence for complex formation. In Figure 3.4, the spectrum of the physical mixture of $\beta$-CD and $\alpha$-tocopherol shows identical resonances with free $\beta$-CD, but a near absence of resonances due to $\alpha$-tocopherol. This behavior has been attributed to the long $T_1$ relaxation times of the guest protons in the physical mixture while the interaction between guest and CD in the inclusion complex shortens this proton $T_1$, and the signals from the guest carbons can be observed (50). In addition, the $\alpha$-tocopherol signals in the physical mixture with $\beta$-CD appear with much less signal intensity due to a lower degree of cross polarization. The dipolar coupling was expected to be reduced by the molecular motion of $\alpha$-tocopherol existing as a liquid when only physically mixed with $\beta$-CD. The cross polarization rate indicates that $\alpha$-tocopherol exists in a more rigid environment having more solid characteristics in the inclusion complex compared to the physical mixture.

The $^{13}$C CP/MAS NMR spectrum of free $\gamma$-CD in Figure 3.5 shows similar behavior of carbon resonance splitting as in $\beta$-CD with a rigid, non-symmetric conformation of the CD crystals. The physical mixture of quercetin dihydrate and $\gamma$-CD appears to have an equivalent spectrum as the individual component spectra, which shows the expected lack of interaction in the physical mixture at the molecular level. The spectrum of the inclusion complex shows most of the major resonances of quercetin and $\gamma$-CD in Figure 3.5. With the presence of quercetin in the $\gamma$-CD cavity of the crystal structure, the resonances of the $\gamma$-CDs show reduced splitting and broadening which indicates the crystals have adopted more symmetric and possibly dynamic conformations. The resonances of the quercetin signals are considerably broadened relative to the spectra of pure quercetin, which suggests that quercetin molecules are in a less ordered, more amorphous environment, which is consistent with true inclusion complex formation. Different crystal lattices of quercetin have been reported between quercetin dihydrate and unhydrated...
quercetin which exhibit themselves in $^{13}$C CP/MAS NMR spectra as a broadening of resonances with release of water from the lattice (25). The observed broadening of the quercetin $^{13}$C resonances in the inclusion complex may be due to the loss of bound crystallization water from the lattice or a change in the conformation of quercetin to a less ordered state.

**Thermogravimetric Analysis.** Thermal analysis has mainly been applied to demonstrate the different behavior of an inclusion compound relative to its physical mixture of component compounds. Table 3.3 confirms that natural antioxidants, $\alpha$-tocopherol and quercetin, have relatively high thermal decomposition temperatures of 280 °C and 329 °C, respectively. It is important to note that thermogravimetric (TG) data only provides weight loss information and oxidative reactions can occur in a sample without observed weight loss (51). The loss of hydration water of each sample was calculated from room temperature to 125 °C. The respective hydration states of $\beta$-CD·10.0 H$_2$O and $\gamma$-CD·7.4 H$_2$O at ambient humidity are in agreement with the reported water vapor sorption isotherms for $\beta$- and $\gamma$-CD at a relative humidity of approximately 30% (52).

Since most CD complexes exist as three-component systems of host:guest:water, it would be expected that upon inclusion of a guest molecule, there would be some degree of displacement of water molecules from the host CD dependent upon the structural fit inside the CD cavity. The 7.3% water content of $\alpha$-tocopherol:$\beta$-CD complex was less than its $\alpha$-tocopherol and $\beta$-CD physical mixture with 11.8%. Quercetin:$\gamma$-CD complexes had a water content of 7.5%, which was less than its quercetin and $\gamma$-CD physical mixture with 9.0%. This TG data is consistent with the inclusion of each guest antioxidant displacing a portion of the original crystalline water molecules present in the CD cavity. The thermograms of both physical mixtures and inclusion complexes were qualitatively different; however, their thermal decomposition temperature did not vary to a great extent (Figures 22-23, 26-27). The inclusion complexes of each natural antioxidant were not observed to have substantially higher thermal decomposition temperatures as is often observed during CD complexation of volatile guests.

**Differential Scanning Calorimetry.** *Enthalpies of Dehydration and Vaporization.* Large increases in resolution of endothermic peaks were obtained by using the pin-hole differential scanning calorimetry (DSC) lid compared to an open container for the measurement of the
vaporization of water (53, 54). The β- and γ-CD hydrates show very broad endothermic peaks from about 25 °C to 160 °C, representing loss of water (Figures 28, 32). Two overlapping peaks were observed in the α-tocopherol:β-CD complex (Figure 3.30) and the physical mixture of quercetin and γ-CD (Figure 3.33), however, the entire peak area was used to calculate the enthalpy of measured effect (ΔH_{meas}) in each case. CD dehydration has an endothermic enthalpic effect which should be considered when studying CD inclusion processes in the solid state. The dehydration step involves breaking the bonds between the CD and water, which is then followed by the vaporization of the freed water molecules.

\[ \beta CD \cdot nH_2O(s) \xrightarrow{\Delta H_{meas}} \beta CD(s) + nH_2O(g) \quad \Delta H_{meas} = 50.1 \text{ kJ/mol} \]

This reaction can be considered as a two-step process:

Dehydration \[ \beta CD \cdot nH_2O(s) \longrightarrow \beta CD(s) + nH_2O(l) \quad \Delta H_{dehyd} = 9.4 \text{ kJ/mol} \]

Vaporization of water \[ nH_2O(l) \longrightarrow nH_2O(g) \quad \Delta H_{vap} = 40.7 \text{ kJ/mol} \]

In Table 3.4, the ΔH_{meas} values are presented with physical mixtures and inclusion complexes adjusted on a weight basis for one gram of CD and by amount for one mole of water due to their respective antioxidant contents. The value for the enthalpy of vaporization (ΔH_{vap}) of water at 100 °C of 40.66 kJ/mol was acquired from handbook data (55). The enthalpy of dehydration (ΔH_{dehyd}) of β-CD obtained from the two-step reaction scheme is 9.4 kJ/mol H₂O. This result is in agreement with the ΔH_{dehyd} of 9.6 kJ/mol H₂O reported by Bilal et al. (56) and the ΔH value of 10.5 kJ/mol H₂O obtained with dissolution enthalpy measurements at 25 °C. An energy input on the order of 10 kJ is required to remove one mole of water from one mole of β-CD.

If the same reaction scheme is applied to β-CD in its physical mixture and inclusion complex with α-tocopherol, the ΔH_{dehyd} obtained are 3.3 and 18.8 kJ/mol H₂O, respectively. The ΔH_{dehyd} of β-CD in the physical mixture was expected to be equivalent to that of β-CD since a host–guest interaction is not present. However, the ΔH_{dehyd} of 18.8 kJ/mol H₂O in the α-tocopherol:β-CD inclusion complex appears to have a sizeable difference from the ΔH_{dehyd} of the free β-CD cavity. This larger ΔH_{dehyd} may be the result of some of the water molecules included within the CD cavity forming hydrogen bonds with the guest molecule (57).
The $\Delta H_{\text{dehyd}}$ of $\gamma$-CD obtained is 6.8 kJ/mol H$_2$O, which is less than $\Delta H_{\text{dehyd}}$ of $\beta$-CD due to $\gamma$-CD having weaker intramolecular hydrogen bonding to hold water molecules. In the CD cavity, the C2 hydroxy group of one glucose unit can form a hydrogen bond with the C3 hydroxy group of the adjacent glucose unit (58). $\beta$-CD has a rather rigid structure due to it forming a complete secondary belt of these hydrogen bonds, while $\gamma$-CD has a noncoplanar, more flexible structure. The strength of hydrogen bonding increases as the CD ring size becomes smaller. This is also evidenced in the DSC curves with $\beta$-CD having a considerably higher endothermic peak temperature of 127.8 ± 1.1 °C compared to $\gamma$-CD with 101.9 ± 3.1 °C which was near the boiling point of water.

The guest quercetin likely exists as a mixture of dihydrate and monohydrate forms at ambient humidity with a calculated 1.7 mol H$_2$O per mol quercetin. The DSC profile of quercetin dihydrate shows a strong endothermic peak at 120.3 ± 1.4 °C for the release of water from the crystal lattice. This temperature is much higher than the boiling point of water which indicates that the water molecules are strongly held by quercetin through hydrogen bonding (25). The $\Delta H_{\text{dehyd}}$ of quercetin is 13.9 kJ/mol H$_2$O. Since quercetin holds bound water in its structure, it was not possible to differentiate the enthalpies of dehydration and vaporization of quercetin from that of its $\gamma$-CD host.

$T_g$ of $\alpha$-Tocopherol. $\alpha$-Tocopherol, in the form of a vitamin E preparation, has been observed to exhibit a glass transition ($T_g$) at approximately -63 °C that was associated with the change from a glassy state to a supercooled liquid (59). In Figure 3.6, DSC curves of $\alpha$-tocopherol and its $\beta$-CD physical mixture both show an endothermic transition characteristic of a $T_g$ at -41 °C. This $T_g$ of $\alpha$-tocopherol was not observed in its complexed form with $\beta$-CD. The absence of thermal events of a guest molecule in a CD complex is generally taken as evidence of true inclusion complexation.

CD complexes of the natural antioxidants, $\alpha$-tocopherol and quercetin, were formed by the coprecipitation technique, which was optimized for complexation yield. ATR/FT-IR, $^{13}$C CP/MAS NMR, TGA, and DSC are powerful and complementary tools for providing evidence of true CD inclusion complexation in the solid state, in addition to observed changes in physical appearance. Natural antioxidant:CD inclusion complexes did not show increased thermal
stability, but may provide increases in oxidative stability. CD complexes of α-tocopherol and quercetin may serve as novel additives in controlled-release active packaging to extend the oxidative stability of foods.

ABBREVIATIONS USED
CD, cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; ATR/FT-IR, attenuated total reflectance/Fourier transform-infrared; CP/MAS, cross-polarization magic angle spinning; Tg, glass transition temperature; TG, thermogravimetric; ΔH<sub>dehyd</sub>, enthalpy of dehydration; ΔH<sub>meas</sub>, enthalpy of measured effect; ΔH<sub>vap</sub>, enthalpy of vaporization; DSC, differential scanning calorimetry

ACKNOWLEDGMENT
This research is based upon work supported by the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) of the National Science Foundation under Agreement No. DGE-0333378. The authors would like to thank Sungsool Wi of the Department of Chemistry for his kind assistance with sample analysis by solid-state NMR.


### Table 3.1. Characteristics of Reactants and Products in the Preparation of Solid, Natural Antioxidant:CD Inclusion Complexes

<table>
<thead>
<tr>
<th>Inclusion Complex</th>
<th>Production Batch</th>
<th>Reactants CD:Antioxidant Molar Ratio</th>
<th>Antioxidant Content wt %&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Product CD:Antioxidant Molar Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol:β-CD</td>
<td>I</td>
<td>8.0</td>
<td>18.1 ± 0.4</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8.0</td>
<td>19.6 ± 0.1</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>8.0</td>
<td>20.2 ± 0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>quercetin:γ-CD</td>
<td>I</td>
<td>7.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.0 ± 0.3</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8.0</td>
<td>15.4 ± 0.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>8.0</td>
<td>14.4 ± 0.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values were reported as mean ± standard error (n = 2)

<sup>b</sup> 21.6 mM γ-CD:3.0 mM quercetin
<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE*&lt;sub&gt;ab&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>27.1</td>
<td>+1.2</td>
<td>+12.7</td>
<td>67.9</td>
</tr>
<tr>
<td>β-CD</td>
<td>94.2</td>
<td>+0.2</td>
<td>+2.1</td>
<td>0.0</td>
</tr>
<tr>
<td>α-tocopherol and β-CD physical mixture</td>
<td>72.1</td>
<td>-0.6</td>
<td>+12.0</td>
<td>24.2</td>
</tr>
<tr>
<td>α-tocopherol:β-CD complex</td>
<td>94.2</td>
<td>-0.4</td>
<td>+3.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Quercetin dihydrate</td>
<td>83.4</td>
<td>-9.0</td>
<td>+48.8</td>
<td>46.4</td>
</tr>
<tr>
<td>γ-CD</td>
<td>91.1</td>
<td>+0.4</td>
<td>+4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Quercetin and γ-CD physical mixture</td>
<td>87.6</td>
<td>-8.1</td>
<td>+39.3</td>
<td>36.4</td>
</tr>
<tr>
<td>Quercetin:γ-CD complex</td>
<td>89.1</td>
<td>-5.3</td>
<td>+21.8</td>
<td>18.8</td>
</tr>
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</table>
Table 3.3. Water Content and Thermal Decomposition Temperature ($T_d$) in Air Atmosphere

<table>
<thead>
<tr>
<th>Sample</th>
<th>H$_2$O</th>
<th>First Stage</th>
<th>Second Stage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt. loss (%)</td>
<td>$T_d$ (°C)</td>
<td>Wt. loss (%)</td>
<td>$T_d$ (°C)</td>
</tr>
<tr>
<td>$\alpha$-tocopherol</td>
<td>0.0</td>
<td>280.2</td>
<td>65.8</td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD</td>
<td>13.7</td>
<td>308.8</td>
<td>72.7</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-tocopherol and $\beta$-CD physical mixture</td>
<td>11.8</td>
<td>236.9</td>
<td>7.0</td>
<td>307.6</td>
</tr>
<tr>
<td>$\alpha$-tocopherol:$\beta$-CD complex</td>
<td>7.3</td>
<td>203.1</td>
<td>1.6</td>
<td>297.5</td>
</tr>
<tr>
<td>quercetin dihydrate</td>
<td>9.2</td>
<td>329.2</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>$\gamma$-CD</td>
<td>9.3</td>
<td>306.5</td>
<td>78.1</td>
<td></td>
</tr>
<tr>
<td>quercetin and $\gamma$-CD physical mixture</td>
<td>9.0</td>
<td>297.4</td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td>quercetin:$\gamma$-CD complex</td>
<td>7.5</td>
<td>163.0</td>
<td>2.2</td>
<td>283.4</td>
</tr>
</tbody>
</table>
Table 3.4. Enthalpy of the Measured Effect ($\Delta H_{\text{meas}}$) and Peak Temperature during Dehydration and Vaporization of Water

<table>
<thead>
<tr>
<th>Sample</th>
<th>H$_2$O</th>
<th>Peak Temperature, °C$^b$</th>
<th>$\Delta H_{\text{meas}}$</th>
<th>$\Delta H_{\text{meas}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol$^a$</td>
<td>1</td>
<td>2</td>
<td>J/g$^b$</td>
</tr>
<tr>
<td>$\alpha$-tocopherol</td>
<td>0.0</td>
<td>------</td>
<td>------</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>$\beta$-CD</td>
<td>10.0</td>
<td>127.8 ± 1.1</td>
<td>------</td>
<td>381.1 ± 15.1</td>
</tr>
<tr>
<td>$\alpha$-tocopherol and $\beta$-CD physical mixture</td>
<td>10.6</td>
<td>120.4 ± 1.4</td>
<td>------</td>
<td>351.8 ± 12.3</td>
</tr>
<tr>
<td>$\alpha$-tocopherol:$\beta$-CD complex</td>
<td>6.1</td>
<td>118.6 ± 2.4</td>
<td>147.5 ± 2.3</td>
<td>291.3 ± 7.0</td>
</tr>
<tr>
<td>quercetin dihydrate</td>
<td>1.7</td>
<td>120.3 ± 1.4</td>
<td>------</td>
<td>278.8 ± 7.9</td>
</tr>
<tr>
<td>$\gamma$-CD</td>
<td>7.4</td>
<td>101.9 ± 3.1</td>
<td>------</td>
<td>245.7 ± 4.6</td>
</tr>
<tr>
<td>quercetin and $\gamma$-CD physical mixture</td>
<td>------</td>
<td>111.8 ± 1.6</td>
<td>122.0 ± 0.1</td>
<td>300.7 ± 15.1</td>
</tr>
<tr>
<td>quercetin:$\gamma$-CD complex</td>
<td>------</td>
<td>112.1 ± 2.1</td>
<td>------</td>
<td>274.1 ± 8.5</td>
</tr>
</tbody>
</table>

$^a$ Number of moles of H$_2$O was calculated from measured weight loss by TGA.

$^b$ Values were reported as mean ± standard error (n = 3).
Figure 3.1. Structure and carbon numbering of the natural antioxidants (a) α-tocopherol and (b) quercetin.
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CHAPTER 4: POLYMER PROCESSING AND CHARACTERIZATION OF LLDPE FILMS CONTAINING α-TOCOPHEROL, QUERCETIN, AND THEIR CYCLODEXTRIN INCLUSION COMPLEXES

ABSTRACT

Natural antioxidant additives were compounded into linear low density polyethylene (LLDPE) using a twin-screw counter-rotating mixer and compression molded into films. Manufactured LLDPE films contained 2715 mg/kg α-tocopherol in its free and β-cyclodextrin complexed form and 1950 mg/kg quercetin in its free and γ-cyclodextrin complexed form. Both cyclodextrin complexes were loaded into films at 1.5% by weight. These natural antioxidants were incorporated into LLDPE resins with two different catalyst types, Ziegler-Natta and metallocene. Films were characterized by optical microscopy, oxidation induction time (OIT), oxygen transmission rate (OTR), contact angle analysis, and atomic force microscopy (AFM). All antioxidant additives increased the oxidative stability of LLDPE as measured by increased OIT, particularly quercetin. Natural antioxidants and their cyclodextrin inclusion complexes may provide a dual function in packaging to protect the polymer from oxidative degradation during melt processing and to delay the onset of oxidation of the packaged food during storage.

KEYWORDS

cyclodextrin, inclusion complex, polymer, α-tocopherol, quercetin, linear low density polyethylene, metallocene catalyst, Ziegler-Natta catalyst, active packaging
INTRODUCTION

During package manufacturing, many antioxidants and stabilizers within a polymer act sacrificially, and are converted into oxidation products as they function to protect the polymer from degradation. The toxicity of these synthetic antioxidant transformation products is either not well known or suspected to be greater than the parent additive (1). Current worldwide trends to avoid or reduce the use of synthetic food additives have given way to investigations of using natural antioxidants in food and packaging systems. Natural antioxidants continue to receive attention in the food industry because of their presumed safety given that they are sourced primarily from plant materials and have been in use for centuries.

The first natural antioxidant incorporated into synthetic polymers was α-tocopherol (2). α-Tocopherol exhibits an excellent melt stabilization to polyethylene and polypropylene at very low concentrations in the range of 100–300 ppm, which surpasses that of the stabilization provided by some of the best synthetic hindered phenol antioxidants traditionally used for this purpose (3). Therefore, α-tocopherol can be employed cost-effectively at only a small fraction of the concentration typically required for polyolefin stabilization by synthetic hindered phenols. α-Tocopherol is also a superior antioxidant for reducing off-odor and off-taste from high density polyethylene (HPDE) bottles compared to the typical synthetic antioxidants, Irganox 1010 and BHT (4-6). The flavonoid antioxidants, such as quercetin, have not been investigated for incorporation into polymer packaging materials.

The natural antioxidants may have a dual function in the polymer packaging to protect the polymer from oxidative degradation during melt processing and to delay the onset of oxidation of the packaged food during storage. Considerable losses of α-tocopherol have been reported in low density polyethylene (LDPE) films likely due to thermo-oxidative effects during extended polymer processing (7, 8). Cyclodextrins ( CDs) are known for their ability to act as host molecules to form inclusion complexes with a wide variety of guest molecules. The advantages of CD inclusion complex formation with natural antioxidants are potential thermo-oxidative protection during polymer melt processing and a provision of controlled release. CD inclusion complexes may be embedded directly into polymer films by melt processing, and the antioxidant is protected both from the environment and its processing into polymers by the thermally stable crystalline lattice provided by the host CD. CDs can decrease the effective guest mobility in a
controlled manner allowing for a continuous, more uniform level of antioxidant release for extended shelf life foods.

Polyethylene is the most important polymer used in food packaging. Linear low density polyethylene (LLDPE) has the fastest growth rate of the three major polyethylene families and is used in the flexible film food market. Polymer additive transport depends on the structural variations and complexity of the polymer backbone in addition to the type and nature of the diffusing additives. Different catalysts in the production of LLDPE can be used to obtain polymers with different molecular weight distributions, crystalline structure morphologies, and diffusion path networking arrangements which may allow differences in additive release rates for active packaging materials.

A more complete understanding of the incorporation of active compounds and their CD inclusion complexes into polymer packaging is necessary to develop and tailor future packaging systems to specific foods. In future applications of this technology, additives will be incorporated into the innermost layer of the film structure, which generally functions as the food contact layer and the sealant layer for the film structure for controlled release to a food product. The purpose of this research was to compound α-tocopherol, quercetin, and their CD inclusion complexes into two catalyst-specific LLDPE resins while maintaining their relative antioxidant activity and stability. LLDPE films loaded with these natural antioxidants will be used in future studies to measure their antioxidant release rates into a model food system.

MATERIALS AND METHODS

Materials. (±)-α-Tocopherol of 98% purity and quercetin dihydrate of 99% purity were supplied by Sigma-Aldrich (St. Louis, MO). Preparation of inclusion complexes of α-tocopherol with β-CD and quercetin with γ-CD was performed as described previously (9). These CD complexes in unhydrated form were stored in a desiccator after the lyophilization stage. Solid α-tocopherol:β-CD complexes with 18.1% (w/w) α-tocopherol content and quercetin:γ-CD complexes with 13.0% (w/w) quercetin content were used as antioxidant additives in LLDPE. HPLC-grade water was obtained from Fisher Scientific (Pittsburgh, PA).

Two commercially available LLDPE resins were selected as comparable materials with different catalyst systems. Ziegler-Natta (ZN) LLDPE had a density of 0.918 g/cm³, C6 comonomer, and a melt flow index of 3.2 dg/min (ASTM D 1238 Condition 190 °C/2.16 kg).
Metallocene (met) LLDPE had a density of 0.918 g/cm³, C6 comonomer, and a melt flow index of 3.5 dg/min (ASTM D 1238 Condition 190 °C/2.16 kg). Both LLDPE resin grades contained a typical antioxidant stabilizer package added by the manufacturer, but no antiblock or slip agents.

The degree of crystallinity (mass fraction, $W_{c,d}$), based on density ($\rho$) was calculated using the following equation (10):

$$W_{c,d} = \frac{1}{\rho_s} - \frac{1}{\rho_a} \times \frac{100}{\rho_c - \frac{1}{\rho_a}} \times \frac{100}{\rho_a},$$

where $\rho_s$, $\rho_c$, and $\rho_a$ are the density of the sample, completely crystalline (1.00 g/cm³), and completely amorphous (0.852 g/cm³) polyethylene, respectively. Both ZN and met LLDPE resins were estimated at 48.6% crystallinity.

**Compounding of Additives into LLDPE.** LLDPE resins were compounded with additives and compression molded into films at the Advanced and Applied Polymer Processing Institute (AAPPI) in Danville, VA. A Plasti-Corder twin-screw counter-rotating mixer type EPL-V5501 (C.W. Brabender Instruments, South Hackensack, NJ) with a measuring head type C.E.E.6-230V8.5AMP, temperature control console type 808-400-DTI, and electronic Plasti-Corder torque rheometer was used to compound antioxidant additives into ZN and met LLDPE resins. Prior to compounding, both LLDPE resin types were purged with a positive pressure of N₂ through the material for at least 24 hours. Concentration levels of natural antioxidants and their inclusion complexes compounded into ZN and met LLDPE resins are summarized in Table 4.1. Resin pellets were added to the 50-mL mixing bowl at a temperature of 190 °C and mixed at a speed of 100 rpm. Control runs were used to determine the time to fusion peak torque and a minimum of five minutes was added to this time to ensure adequate and equal mixing time in melt shear. The torque rheometer was monitored to assess the time to peak fusion torque and estimate the melt viscosity. The residence time at 190 °C and 100 rpm was 5 min. A N₂ bleed was applied to the opening of the small bowl during shear mixing of LLDPE and additives. Three compounded batches of each additive treatment and two control batches of each LLDPE resin type were produced. Control batches received the same thermal exposure by melting and mixing the resin pellets. A purge material was used to clear the bowl between different additive treatments. Each compounded LLDPE batch in the melt state was manually pressed between
two polytetrafluoroethylene (PTFE) coated platens into preforms of <10 mm thickness and approximately 43 g weight for subsequent compression molding.

**Compression Molding of LLDPE into Films.** The compression molding method was modified from ASTM D 4703-03 (11). A compression molding press Genesis Series G100H-18-CX (Wabash MPI, Wabash, IN) was used to compression mold the preforms of LLDPE into films. A brass picture frame mold with inner dimensions of 23.5 cm × 23.5 cm and thickness of 820 µm was used with aluminum foil (20-µm thickness) as the parting agent. The mass of the preform was sufficient to fill the mold volume (45.3 cm³) when it was melted and provided an excess of about 3% (w/w) for flash. Manually pressed LLDPE preforms were placed in the picture-frame mold between two, aluminum foil parting agents and then the assembly was inserted between the PTFE-coated platens. The mold assembly was preheated between both platens and set at a process temperature of 190 °C by closing the press with application of contact pressure for 5 min. The press platens at 190 °C were closed with 6 tons of ram force in three cycles to remove entrapped air in the material and prevent the development of voids in the films: (1) 10-s press, (2) 10-s press, and (3) 5-min press. At the low pressure of approximately 140 psi, the presence of any entrapped air was more easily observed. Films were cooled by air, followed immediately by circulated water cooling under compression to 40 °C at an average cooling rate of 23 °C/min. The press was opened and films were removed from the mold at a temperature <40 °C. LLDPE films containing each antioxidant additive and control films of each resin type were produced in triplicate and duplicate, respectively. Films were punched with a 45-mm diameter steel blanking die resulting in 45.2-mm diameter sample discs to perform future controlled-release studies. LLDPE film thickness of 10 sample discs from four different batches were measured with a micrometer at four locations across the film as 870 ± 2 µm (n = 160). All sample films were stored at refrigeration temperatures and protected from light to minimize additive migration and maintain additive stability.

**Optical Transmission Microscopy.** An Eclipse series 80i advanced research microscope (Nikon Instruments, Melville, NY) with a DS-Fi1 digital imaging head and CFI Plan Fluor series objective lens at 20× magnification was used to observe particulate antioxidant additives and
their qualitative degree of dispersion in LLDPE films. NIS Elements Imaging Software BR 2.30 (Nikon Instruments, Melville, NY) was used to process images.

**Oxidation Induction Time.** LLDPE films with additives were cut into 3.2-mm diameter discs of $870 \pm 2 \, \mu m$ thicknesses using a bore-hole cutter to obtain sample weights of $6.5 \pm 0.2 \, mg$. Sample discs were placed in an open standard aluminum differential scanning calorimetry (DSC) pan. A DSC Q1000 instrument (TA Instruments, New Castle, DE) was used with a sample purge flow of $N_2$ at 50 mL/min. The DSC was equilibrated at 50 °C, ramped to 200 °C at 20 °C/min, held isothermal for 5 min, and then the gas was switched to $O_2$ at 12.5 min. Samples were held under $O_2$ isothermally at 200 °C until stopping the run after the maximum slope of the exotherm was observed in the real-time DSC curve. Universal Analysis 2000 (TA Instruments, New Castle, DE) software was used to analyze the oxidation induction time (OIT) of each sample ($n = 2$) by the tangent method. This method follows ASTM Standard D 3895-04 (12).

**Oxygen Transmission Rate.** An 8001 oxygen permeation analyzer (Illinois Instruments, Johnsburg, Illinois) was calibrated at 23.0 °C and 0% relative humidity with a standard Mylar film of known oxygen transmission rate (OTR) of 64.8 cm³/m²/day. $N_2$ flow through the bottom chamber was 19 cm³/min and $O_2$ flow through the top chamber was 20 cm³/min. A masking plate with a diameter of 2.5 cm (4.91 cm² surface area) was used. LLDPE control and antioxidant additive films ($n = 2$) of $870 \pm 2 \, \mu m$ thickness were measured for OTR (cm³/m²/day) at 23.0 °C, 0% relative humidity, and atmospheric pressure until an equilibrium condition was present at 360 min.

**Contact Angle Measurements.** Static water contact angle measurements were performed at room temperature using a FTA200 dynamic contact angle analyzer (First Ten Angstroms, Portsmouth, VA) connected to a B/W CCD camera (Sanyo North America, San Diego, CA) above a benchtop vibration isolation system with Stabilizer Technology (Newport, Irvine, CA). A constant sessile droplet volume of water was used and delivered to the surface of each LLDPE film by an 18-gauge needle with a pump out rate of 0.20 µL/s. Contact angles of water droplets with good symmetry were reported as a mean of the left and right angles on control LLDPE films ($n = 6$) and LLDPE films with antioxidant additives ($n = 9$).
**Atomic Force Microscopy.** A MultiMode scanning probe microscope (Veeco Instruments, Santa Barbara, CA) with a type E piezoelectric scanner was used at ambient temperature to image the surface morphology of selected LLDPE films. Antimony (n) doped silicon probes (Veeco model TESP7) with nominal tip radius of 8 nm, force constant of 42 N/m, and resonance frequency of 320 kHz were used. LLDPE film samples with dimensions of 10 mm × 10 mm were scanned in several different areas to obtain a representative surface image. The atomic force microscope (AFM) was operated in tapping mode with a scan area of 3.0 × 3.0 µm², a scan rate of 1.0 Hz, and 512 lines per image. The free air amplitude (A₀) and set-point amplitude (Aₛₚ) were set at target values of 6.5 and 4.5 volts, respectively. Therefore, the set-point amplitude ratio (rₛₚ) represented moderate tapping with a value of 0.7. Raw height and phase images were processed for background removal (flattening) by using the MultiMode's image analysis and presentation software.

**Statistical Analysis.** Statistical analysis of the antioxidant additives in each individual LLDPE resin type was performed by a one-way analysis of variance with the general linear model supported by SAS (Version 9.1.3, 2003, SAS, Cary, NC). Multiple comparisons were adjusted for the Tukey-Kramer method of the general linear model procedure to test for least-squares mean separation. Effects were considered significant at p < 0.05. Data values were reported as mean ± standard error.

**RESULTS AND DISCUSSION**

**LLDPE Resin Systems.** Multiple-site catalysis of polyethylene leads to polymer chains with varying structures across the chains and a broad molecular weight distribution. Conventional multi-site Ziegler-Natta (ZN) LLDPEs are characterized by broad molecular weight distributions and broad comonomer distributions. ZN LLDPE has more comonomer in the lower molecular weight fraction and less in the high molecular weight fraction (13). Single-site catalyzed LLDPE has comonomer much more uniformly distributed along the polymer backbone than do conventional resins. Metallocene (met) catalysts are highly active catalysts exhibiting an exceptional ability to polymerize olefin monomers, and have advantages over the conventional ZN catalysts. Metallocenes produce extremely uniform polymers and copolymers of narrow molecular weight distribution and narrow comonomer distribution, and simultaneously control
the resulting polymer chain architectures. A narrower comonomer distribution results in a narrower crystallite size distribution in the crystalline morphology. This narrower crystalline size distribution will have a significant impact on additive diffusion and release rate through the material. Control of crystalline morphology translates into control of diffusion path tortuosity. Therefore, this study compares a ZN LLDPE and a met LLDPE having the same density (crystallinity), but different crystalline structure morphologies and diffusion path networking arrangements that may allow for differences in release rate. LLDPE resins were selected after surveying the patent literature (14-18), which describes a range of recommended and preferred resin characteristics for packaging film sealant layers. Regardless of whether the sealant layer polyethylene copolymer is produced by heterogeneous catalysis (ZN LLDPE) or homogeneous catalysis (met LLDPE), the preferred range for the melt index is 1.3–8.1 dg/min (ASTM D 1238 Condition 190 °C/2.16 kg) and a density within the range of 0.88–0.94 g/cm³. The crystallinity of LLDPE films was assumed to be equivalent based on the commercial supplier density specifications and the films experiencing the same compounding and compression molding conditions. The role of crystallinity may be investigated further by performing crystallization half-time experiments.

LLDPE is a saturated hydrocarbon that has a generally stable structure. Tertiary carbons at short-chain branch points and double bonds at chain ends are the most reactive parts of the polymer molecule due to its method of manufacture (13). The conversion of resin pellets into packaging films or containers involves both high temperature and mechanical force on the polymer in the melt state. The shearing of the polymer chains produces macroradicals that react with oxygen to produce hydroperoxides. As a result, extensive mechano-oxidation of the polymer occurs if the subsequent chain reaction is not inhibited at this point. Oxygen-containing low molecular weight compounds, such as aldehydes and ketones, can form and become entrapped in the polymer matrix followed by evaporation in air as off-odor volatiles or migration to food as off-taste compounds. Therefore, processing antioxidants and stabilizers are added to the polymer before the extrusion or molding process in an effort to limit hydroperoxide formation (1). While it is impossible to exclude oxygen during processing, this study aimed to reduce such effects by nitrogen purging the LLDPE resins and using a nitrogen bleed over the mixing bowl during compounding. These LLDPE resins also contained the typical commercial stabilizer package in addition to the compounded natural antioxidant additives.
Torque readings were measured continuously and provided an estimate of the melt viscosity for the two LLDPE resin types. Control ZN LLDPE had a maximum torque of 1450 m-g at initial time with an end torque of 1130 m-g after 5 min. Control met LLDPE had a maximum torque of 1510 m-g at initial time with an end torque of 1250 m-g after 5 min.

Physical Appearance of Additive-Loaded LLDPE Films. All natural antioxidants and their CD complexes were observed to be very uniform and consistent throughout the LLDPE without any particulates observed on the macroscale (Figure 4.4). The manufactured ZN and met LLDPE films contain 2715 mg/kg α-tocopherol in its free and β-CD complexed form, and 1950 mg/kg quercetin in its free and γ-CD complexed form as summarized in Table 4.1. At 0.20 and 0.27% natural antioxidant concentrations, these are at the typical concentration levels used with commercial synthetic hindered phenols (19).

Control met LLDPE films are observed to have greater clarity than the conventional ZN LLDPE control. Free α-tocopherol and its β-CD complex loaded into LLDPE films exhibit a very slight amber color. Films loaded with quercetin are observed as an intense yellow color, but this yellow intensity is noticeably subdued in films containing the quercetin:γ-CD complex.

Additive Dispersion in LLDPE Films. The relatively polar structure of the CD molecule has presented difficulties in distributing this additive and its inclusion complexes into nonpolar matrices, such as polyolefins. Siró et al. (20) observed the visible inhomogeneous distribution of α-tocopherol:β-CD complexes in LDPE film. Their materials were processed with a single-screw extruder and were observed to have CD agglomerates that appeared as white spots in the LDPE matrix. Single-screw extrusion has also produced LDPE films containing uncomplexed β-CD in the form of aggregates (21). Other processing techniques, including melt pressing and solution casting, appear to have limited effectiveness in uniformly distributing inclusion complexes throughout the polymer (22).

In this study, the dispersion of natural antioxidant:CD complexes within LLDPE was optimized by mixing the resin and additive in the melt state with a twin-screw counter-rotating mixer. No visual aggregation of additives was observed as had been reported by previous researchers, which indicates that polymer and additive were mixed efficiently and only additive solubility within the polymer was a limiting factor. The solubility of additives is dependent upon
the volume of amorphous fraction and the polymer structure. Many additives are compounded into the polymer at levels above their intrinsic solubility and, therefore, exist in a supersaturated solution. Such additives have low diffusion coefficients and migration is thereby minimized, or a two-phase system is formed which consists of a saturated solution of additive in polymer and a separate additive phase.

Additives dissolved in a polymer are primarily located in the amorphous phase in zones around knots, folds and various chain entanglements where there is sufficient free volume to hold the molecule (23). Uniform dispersion of the antioxidants during compounding is critical for obtaining optimal performance of higher molecular weight antioxidants, which are expected to have relatively slow diffusion rates. The temperature of processing the polymer may play an important role in the type of dispersion obtained (24). Quercetin has a melting point (323 °C (25)) above the process temperature of 190 °C, and its resulting dispersion is of the particulate type, in which quercetin exists as separate phases in the LLDPE matrix. α-Tocopherol exists as a liquid at room temperature so its melting temperature is clearly below 190 °C, and it forms a more solution-like dispersion in which α-tocopherol molecules are homogeneously dispersed in the polymer matrix.

**Optical Transmission Microscopy.** The LLDPE films containing natural antioxidant additives were viewed under 200× magnification to obtain a qualitative measure of each additive solubility in the bulk polymer. The α-tocopherol and α-tocopherol:β-cyclodextrin complex were not observed throughout the thickness of the LLDPE films, which indicates that these additives were both soluble in the amorphous phase. This observation was rather remarkable since the relatively polar β-CD host molecule is not miscible within the LLDPE (27). The α-tocopherol:β-CD complex appears to have much less polarity than its free β-CD host, which was indicated previously by its lack of water solubility (9). α-Tocopherol also may have retained its ability to act as a plasticizer from within its β-CD inclusion complex thereby increasing the additive solubility.

Figure 4.1 shows evenly distributed composites of both quercetin and quercetin:γ-cyclodextrin complex in LLDPE, indicating their insolubility in the amorphous phase. These films possess a discernable phase boundary between the polymer bulk and the domains of both quercetin and its γ-CD complex. Surface antioxidant concentrations were observed to be
equivalent compared to concentrations in the bulk LLDPE, qualitatively indicating that there was not an affinity for the polymer–air interface. Quercetin appears as opaque short, needle-like crystals. The crystal structure of quercetin exists as hydrogen-bonded dimers connected by water molecules (26). In contrast, quercetin’s $\gamma$-CD complex appears as translucent, thin, tetragonal crystals. $\gamma$-CD inclusion complexes are known to form channel-type crystalline structures with either tetragonal or hexagonal packing (27, 28). LLDPE films loaded with quercetin: $\gamma$-CD complex are of lighter yellow intensity than the equivalent weight percent of quercetin. This reduction in color intensity may be explained by the presence of quercetin within the crystalline channel structure of $\gamma$-CD.

**Oxidation Induction Time.** The oxidation induction time (OIT) method is an antioxidant performance test that is also useful for characterizing the degree of dispersion of antioxidants (24). In polymer matrices containing fairly uniform antioxidant dispersions, the OIT increases approximately linearly with increasing antioxidant concentration. The OIT is a measure of when the onset of oxidation of the polymer matrix occurs. The OIT is generally considered the time at which the active antioxidant in the polymer has been completely consumed.

Antioxidant performance is expected to improve as the antioxidant becomes more uniformly dispersed in the polymer matrix because in order to inhibit the oxidation reaction, the additive must chemically contact the peroxyradical on the polymer chains (24). The contact between the dispersed additive and the peroxyradical occurs by diffusion processes. As the degree of dispersion of the antioxidant increases, the average required diffusion path length and, therefore, diffusion time is shortened. The termination reaction rate of immobile macroradicals attached to the polymer chain is directly proportional to the concentration of mobile antioxidant molecules (23). At a constant average antioxidant concentration, the OIT increases as the degree of dispersion increases.

In Table 4.2, the OIT of the control films at 200 °C vary considerably between ZN and met LLDPE with values of 24.4 ± 0.4 and 81.4 ± 1.1 min, respectively. These commercial resin grades contain a typical antioxidant stabilizer package, but differences in OIT may be due to differences in polymerization processes and residual metal catalysts. The antioxidant concentrations of 2715 mg/kg $\alpha$-tocopherol and 1950 mg/kg quercetin in LLDPE are nearly equivalent on a molar basis and all additives resulted in increased OIT of the polymer. Free $\alpha$-
tocopherol provides considerable additional oxidative protection to ZN and met LLDPE by extending the OIT by 59.3 and 14.8 min, respectively. The β-CD complex of α-tocopherol is even more effective than free α-tocopherol with the OIT extended by 68.1 and 38.9 min in ZN and met LLDPE, respectively. This added protection of the β-CD complex is either because α-tocopherol is stabilized during polymer processing or experiences increased antioxidant effectiveness within the CD cavity.

Quercetin exhibited excellent performance by extending the OIT of ZN and met LLDPE by 128.4 and 124.7 min, respectively. This result is remarkable since quercetin exists as a particulate dispersion in LLDPE. Several studies have shown that antioxidants, including quercetin (29, 30), can function effectively in suspension in bulk vegetable oils (31). The reported solubility limit of quercetin in soybean oil is approximately 100 µM (34 mg/L) (32), which is likely on the order of its solubility in polyethylene. The activity of partially soluble, suspended quercetin may be attributed to its more favorable orientation at the air-polymer interface where surface oxidation occurs (31). Low solubility with an effective antioxidant is not a disadvantage if the diffusion or dissolution rates are not determining factors (33). The quercetin:γ-CD complex extended the OIT by 12.2 and 44.4 min in ZN and met LLDPE films, respectively, relative to their controls. The performance of quercetin in its γ-CD complex has been greatly suppressed relative to quercetin in its free form, but remains comparable to the α-tocopherol:β-CD complex in met LLDPE. In its γ-CD complex, quercetin is expected to have a lower diffusion coefficient due to a large increase in molecular weight from the γ-CD host molecule. Such a decrease in mobility would not allow quercetin to contact macroradicals and peroxyradicals on the polymer chains as effectively.

**Oxygen Transmission Rate.** Oxygen transmission rate (OTR) was measured as an indicator of antioxidant additive location within the free volume of the amorphous phase of the polymer. In Table 4.3, LLDPE films were observed to have high oxygen permeability as expected with both ZN and met types having respective OTR values of 163.0 ± 6.0 and 150.5 ± 0.5 cm³/m²/day. The relatively broad molecular weight distribution of ZN LLDPE results in more overall mobility of molecules, which may have contributed to this slightly higher OTR. α-Tocopherol is soluble in the LLDPE and is likely to plasticize the film, which increases the
diffusion coefficient for oxygen. This plasticizing effect is small since the α-tocopherol loading is at a low level of 0.28% (w/w), and may be observed in ZN LLDPE with an increase in OTR to 173.5 ± 0.5 cm³/m²/day. Wessling et al. (8) observed a trend of increasing OTR through LDPE films containing increasing levels of α-tocopherol.

Additives in the form of platelets can lower the permeability more than additives of compact geometries. Platelets that are oriented in the plane of the polymer film will reduce the effective diffusion coefficient by creating a tortuous path for the oxygen molecules traversing the film. Quercetin loaded into LLDPE at 0.20% (w/w) resulted in improved oxygen barrier properties with decreased OTR values of 147.5 ± 2.5 and 129.5 ± 3.5 cm³/m²/day in ZN and met LLDPE, respectively. Free CDs have been reported to exhibit improved barrier properties of diffusion and transmission rate when dispersed in HDPE films (34). CD complexes of both α-tocopherol and quercetin loaded at the 1.5% (w/w) level resulted in decreased OTR values in met LLDPE, but no difference in ZN LLDPE films.

**Contact Angle Measurements.** Static water contact angle experiments were performed on control and loaded LLDPE films to characterize the natural antioxidant additives’ effect on the hydrophobicity of the polymer surface. In Table 4.4, the LLDPE control films had water contact angles of 94.2° ± 0.8 and 92.9° ± 0.7 for ZN and met LLDPE, respectively. The mean water contact angles across all antioxidant additive treatments were significantly different between the ZN (96.3°) and the met (94.1°) LLDPE. The topography of the surface roughness can strongly influence the configuration of the three-phase contact line between the solid, air, and liquid droplet (35, 36). The optical microscopy images created the expectation that both quercetin and its γ-CD complex would have greater contact angles due to the increased surface roughness of these additives as particulate dispersions in LLDPE. Increasing water contact angle indicates either increased hydrophobicity or greater roughness imparted to the polymer surface by the additives. In ZN LLDPE films, the water contact angles for both α-tocopherol:β-CD complex and quercetin loadings were significantly greater than those for the control. In met LLDPE films, the quercetin loadings had a significantly higher contact angle than its control.

**Atomic Force Microscopy.** Atomic force microscopy (AFM) image interpretation requires great care since the image is not solely a function of the sample surface, but represents a
convolution of the forces between the tip and surface. Very small sections of the macroscopic surface are scanned which is not necessarily representative of the entire sample. The structure and morphology of the uppermost polymer surface layers, usually several nanometers thick, generally differ from those of the bulk (37). The height profile provides an estimate of the nature of the surface roughness. In Figure 4.2, the height images of both LLDPE control films do not show much variation, and the surface topography of linear striations is simply due to the aluminum foil parting agent used during compression molding. The height images of ZN LLDPE loaded with α-tocopherol and its β-CD complex in Figure 4.3 show an apparent increase in surface roughness on the film.

Phase imaging provides a method of distinguishing surface features of different moduli. The phase images provide a map of stiffness variation on the sample surface such that a stiffer region has a more positive phase shift and, therefore, appears brighter in a phase image (38). In Figure 4.2, the surface morphology of both neat LLDPE films appears to exist as a fringed micelle model. Small crystallites and amorphous phases co-exist in the semicrystalline LLDPE. The long molecular chains meander from one micelle to another, passing through amorphous regions, and the fringes represent transition materials between the crystalline and amorphous phases (39). The darker regions of the phase image correspond to the LLDPE’s softer, amorphous phase while the brighter regions correspond to the surface lamellae. In Figure 4.3, both ZN LLDPE surfaces were observed to be partially covered with natural antioxidant additive, but the lamellae and amorphous regions can still be observed between gaps of the additive. These results are generally similar to AFM images showing surface coverage of the slip agent erucamide on LLDPE films (40).

Natural antioxidants and their CD complexes were successfully incorporated into LLDPE films in a uniform distribution without any visual aggregation of these additives. All antioxidant additives increased the oxidative stability of LLDPE, notably the flavonoid quercetin. The flavonoids have not previously been investigated as polymer additives, but they appear to have great potential to stabilize polymers from oxidative degradation if their added color is either not a concern or desired as a natural colorant. Natural antioxidants could fill the widespread need for additives that can be used in a variety of direct food contact applications or packaging. These natural antioxidant additives may have a dual function in the packaging to protect the polymer
from oxidative degradation during melt processing and to delay the onset of oxidation of the packaged food during storage.

ABBREVIATIONS USED
CD, cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; LLDPE, linear low density polyethylene; met, metallocene; ZN, Ziegler-Natta; PTFE, polytetrafluoroethylene; DSC, differential scanning calorimetry; OIT, oxidation induction time; OTR, oxygen transmission rate; AFM, atomic force microscopy

ACKNOWLEDGMENT
This research is based upon work supported by the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) of the National Science Foundation under Agreement No. DGE-0333378. The authors would like to thank Ronald D. Moffitt at AAPPI for access to equipment for polymer processing.
LITERATURE CITED


**Table 4.1. Concentration Levels of Natural Antioxidant Additives Compounded into LLDPE**

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>Concentration Level</th>
<th>Concentration Level</th>
<th>Concentration Level</th>
<th>Concentration Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antioxidant in LLDPE</td>
<td>Additive in LLDPE</td>
<td>Additive in LLDPE</td>
<td>Antioxidant in LLDPE</td>
</tr>
<tr>
<td></td>
<td>wt. %</td>
<td>wt. %^a</td>
<td>mg/kg^a</td>
<td>mg/kg^a</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>98.0</td>
<td>0.28</td>
<td>2770</td>
<td>2715</td>
</tr>
<tr>
<td>α-tocopherol:β-CD complex</td>
<td>18.1</td>
<td>1.50</td>
<td>15000</td>
<td>2715</td>
</tr>
<tr>
<td>Quercetin</td>
<td>99.0</td>
<td>0.20</td>
<td>1970</td>
<td>1950</td>
</tr>
<tr>
<td>Quercetin:γ-CD complex</td>
<td>13.0</td>
<td>1.50</td>
<td>15000</td>
<td>1950</td>
</tr>
</tbody>
</table>

^aNatural antioxidant concentrations listed are in addition to the typical stabilizer system in commercial LLDPE resins.
Table 4.2. Oxidation Induction Time (OIT) of LLDPE Films with Antioxidant Additives at 200 °C

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>OIT (min) &lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ziegler-Natta LLDPE</th>
<th>Metalloocene LLDPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>24.4 ± 0.4 e</td>
<td>81.4 ± 1.1 e</td>
<td></td>
</tr>
<tr>
<td>α-tocopherol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.7 ± 0.5 c</td>
<td>96.2 ± 0.8 d</td>
<td></td>
</tr>
<tr>
<td>α-tocopherol:β-CD complex&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.5 ± 1.4 b</td>
<td>120.3 ± 1.3 c</td>
<td></td>
</tr>
<tr>
<td>quercetin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.8 ± 0.0 a</td>
<td>206.1 ± 0.9 a</td>
<td></td>
</tr>
<tr>
<td>quercetin:γ-CD complex&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.6 ± 0.3 d</td>
<td>125.8 ± 0.4 b</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Values were reported as mean ± standard error (n = 2)

<sup>b</sup> 2715 mg/kg α-tocopherol

<sup>c</sup> 1950 mg/kg quercetin
Table 4.3. Oxygen Transmission Rate (OTR) of LLDPE Films with Antioxidant Additives

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>OTR (cm$^3$/m$^2$/day)$^a$</th>
<th>Ziegler-Natta LLDPE</th>
<th>Metallocene LLDPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>163.0 ± 6.0</td>
<td>150.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-tocopherol$^b$</td>
<td>173.5 ± 0.5</td>
<td>147.0 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>$\alpha$-tocopherol:β-CD complex$^b$</td>
<td>160.0 ± 7.0</td>
<td>139.0 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>quercetin$^c$</td>
<td>147.5 ± 2.5</td>
<td>129.5 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>quercetin:γ-CD complex$^c$</td>
<td>159.5 ± 2.5</td>
<td>133.5 ± 6.5</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values were reported as mean ± standard error (n = 2)

$^b$ 2715 mg/kg $\alpha$-tocopherol

$^c$ 1950 mg/kg quercetin
Table 4.4. Static Water Contact Angle Measurements of LLDPE Films with Antioxidant Additives

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>Water Contact Angle (deg)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ziegler-Natta LLDPE</td>
</tr>
<tr>
<td>control</td>
<td>94.2 ± 0.8  b</td>
</tr>
<tr>
<td>(\alpha)-tocopherol\textsuperscript{b}</td>
<td>96.2 ± 0.6  ab</td>
</tr>
<tr>
<td>(\alpha)-tocopherol:(\beta)-CD complex\textsuperscript{b}</td>
<td>97.2 ± 0.7  a</td>
</tr>
<tr>
<td>quercetin dihydrate\textsuperscript{c}</td>
<td>97.1 ± 0.5  a</td>
</tr>
<tr>
<td>quercetin:(\gamma)-CD complex\textsuperscript{c}</td>
<td>96.8 ± 0.8  ab</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values were reported as mean ± standard error for control (n = 6) and antioxidant additives (n = 9).

\textsuperscript{b} 2715 mg/kg \(\alpha\)-tocopherol

\textsuperscript{c} 1950 mg/kg quercetin
**Figure 4.1.** Optical transmission microscopy of (a) quercetin and (b) quercetin:γ-cyclodextrin inclusion complex localization within Ziegler-Natta LLDPE at 200× magnification.
Figure 4.2. Atomic force microscopy height (left) and phase (right) images (3.0 µm²) of (a) Ziegler-Natta LLDPE and (b) metallocene LLDPE. In both height images, the contrast covers variations in the 0–50 nm range. In the phase images, the contrast covers phase shifts of (a) 30° and (b) 20°.
Figure 4.3. Atomic force microscopy height (left) and phase (right) images (3.0 μm²) of (a) α-tocopherol and (b) α-tocopherol:β-CD complex in Ziegler-Natta LLDPE. In the height images, the contrast covers variations in the (a) 0–100 nm and (b) 0–50 nm range. In the phase images, the contrast cover phase shifts of (a) 30° and (b) 70°.
APPENDIX

Figure 4.4. (a) Ziegler-Natta and (b) metallocene LLDPE films containing natural antioxidant additives. Visual appearance of optical translucence and color indicated by logo design with black text and white background.
**Figure 4.5.** Example of water contact angle characterization on the surface of a metallocene LLDPE control film.
Figure 4.6. Example of oxidation induction time (OIT) determination of Ziegler-Natta LLDPE containing 1950 mg/kg quercetin by DSC.
CHAPTER 5: CONTROLLED RELEASE OF \( \alpha \)-TOCOPHEROL AND ITS \( \beta \)-CYCLODEXTRIN INCLUSION COMPLEX FROM LLDPE INTO COCONUT OIL

ABSTRACT

Polymer additive migration into a food product is dependent upon numerous factors including the original concentration of the additive in the polymer, its solubility in the food, its partition coefficient between the polymer and food, temperature, and time. The limited solubility of quercetin in linear low density polyethylene (LLDPE) did not allow release from the film due to phase segregation of the quercetin in the bulk polymer. Increasing the molecular weight of \( \alpha \)-tocopherol by \( \beta \)-cyclodextrin (CD) complexation can greatly reduce its diffusion coefficient in LLDPE. Ziegler-Natta and metallocene LLDPE contain different crystalline structure morphologies and diffusion path networking arrangements that allow for differences in additive release rates. Effective controlled-release packaging should combine CD complexation of additives and polymer morphology control to target delivery of an optimal antioxidant concentration to achieve prolonged activity, resulting in extended shelf life foods.

KEYWORDS
cyclodextrin, diffusion coefficient, inclusion complex, migration, natural antioxidant, \( \alpha \)-tocopherol, quercetin, linear low density polyethylene, active packaging
INTRODUCTION

Active packaging is primarily designed to prolong shelf life, improve safety, and enhance sensory properties in foods and beverages. The reliance on active packaging technologies continues to grow as consumers demand fresh, high quality, and convenient food products. Oxidation is the most serious problem the food industry faces in protecting shelf-stable foods due to its deteriorating effects on food quality. The major food quality issues influenced by lipid oxidation include nutritional quality, toxicity, flavor, texture, and color (1). Antioxidants are added to foods to intercept and react with free radicals at a rate faster than the lipid substrate.

The current incorporation of antioxidants throughout the entire food matrix in one large initial dose is not an efficient process due to oxidation occurring at the surface and high initial doses of antioxidant having prooxidant effects. The oxygen necessary to promote lipid oxidation typically diffuses from the food surface into the interior and, therefore, oxidation is initiated at the food surface. The use of active packaging to attain a controlled release rate of antioxidants would be ideal to inhibit the oxidative reaction directly on the food surface. Natural antioxidants may exhibit prooxidant behavior which is particularly dependent upon antioxidant concentration level. α-Tocopherol provides its greatest antioxidant activity at lower concentrations and either decreases or exhibits prooxidant activity at higher concentrations in bulk vegetable oils. The optimal concentration for α-tocopherol to exhibit greatest antioxidant potency is approximately 100 ppm in corn (2), soybean (3, 4), and fish (5) oils. Most of the phenolic acids, flavonoids, anthocyanidins, and anthocyanins exhibit some prooxidant activity at very low concentrations (6). The prooxidant effect of flavonoids is most pronounced at very low antioxidant concentrations (<1–2 µM), and is reversed at higher concentrations (7). Controlled-release packaging also may allow the targeting of a constant antioxidant concentration dependent upon the specific antioxidant and food system to achieve optimal, prolonged activity.

Controlled-release active packaging means both a prolonged duration of active additive delivery and predictability and reproducibility of release rates. Synthetic antioxidants, such as BHT and BHA, have been successfully delivered from high density polyethylene packaging to slow the oxidation of oatmeal cereal (8). Synthetic antioxidant-incorporated polyethylene films in contact with vegetable oils and fish muscle also show protective effects from lipid oxidation (9, 10). However, the release rates of synthetic antioxidants from polymer packaging are significantly more rapid than that of α-tocopherol due to their relatively high diffusion
coefficients (8, 11-13). Cyclodextrins (CDs) can function to decrease the effective antioxidant mobility by formation of inclusion complexes. The incorporation of CD complexes of natural antioxidants into polymer packaging aims to decelerate the release of active antioxidant to provide a continuous replenishment throughout the entire shelf life of the packaged food.

CD complexes of the natural antioxidants, \( \alpha \)-tocopherol and quercetin, have been prepared previously and incorporated into linear low density polyethylene (LLDPE) films of two different catalyst types in an earlier study (14). The purpose of this research was to measure the release kinetics of \( \alpha \)-tocopherol and quercetin in their free and CD inclusion complexed forms from LLDPE films into a coconut oil model system. A mathematical model was applied to estimate diffusion coefficients and active concentration of antioxidant within the polymer films. Food packaging containing CD complexes of antioxidants will allow another packaging innovation to be available to food and packaging technologists, which may prove useful in a hurdle approach to inhibit oxidative processes in extended shelf life products.

MATERIALS AND METHODS

**Materials.** (±)-\( \alpha \)-Tocopherol of 98% purity and quercetin dihydrate of 99% purity were supplied by Sigma-Aldrich (St. Louis, MO). Potassium phosphate monobasic (KH\(_2\)PO\(_4\)), methanol, tetrahydrofuran (THF), acetonitrile, isopropanol, and water of HPLC grade, and 99.5% ethanol of ACS reagent grade were obtained from Fisher Scientific (Pittsburgh, PA). n-Hexanol (98.5%) was received from Sigma-Aldrich (St. Louis, MO). Ethanol (95%) was prepared by dilution of 99.5% ethanol in HPLC-grade water. Refined, bleached, and deodorized coconut oil was kindly donated by Archer Daniels Midland (Quincy, IL) with a measured peroxide value of 0 meq O\(_2\)/kg. Ziegler-Natta (ZN) and metallocene (met) LLDPE films loaded with 2715 mg/kg \( \alpha \)-tocopherol in its free and \( \beta \)-CD complexed form, and 1950 mg/kg quercetin in its free and \( \gamma \)-CD complexed form were prepared as described previously (14).

**Coconut Oil Model System.** Clear glass straight-sided jars (60 mL) with polytetrafluoroethylene faced polyethylene-lined caps (Fisher Scientific, Pittsburgh, PA) were used as migration test cells with an inner diameter of 48.8 mm and a total volume of 74.5 mL. An 8% (w/v) LLDPE film to coconut oil ratio was selected to allow sufficient antioxidant release to target optimal \( \alpha \)-tocopherol activity in vegetable oil and to have the same scale as polymer
material in a commercial vegetable oil package which was 6% (w/v). LLDPE sample films with 45.2 mm diameter and weight of 1.30 ± 0.005 g were submerged in 15.0 mL coconut oil in each test cell and placed in a PsycroTherm (model G-26) controlled environment incubator shaker (New Brunswick Scientific, Edison, NJ) at 30.0 ± 0.5 °C and 100 rpm protected from light. An air headspace volume of 58.1 mL was present in each test cell. Samples (n = 3) were removed for quantification of antioxidant concentration by HPLC at 0, 1, 3, 7, 14, 21, and 28 days of storage time. The same experimental setup was used for 95% ethanol as a food simulant (n = 3).

**Density, Viscosity, and Peroxide Value of Coconut Oil.** A coconut oil sample was equilibrated at 30 °C in a water bath. The density of coconut oil at 30 °C was determined by a pycnometer. Water was used as the working liquid with a known standard density at 30.0 °C of 0.9956511 g/cm³ (15). The following equation was used to solve for the density of coconut oil (ρ_{oil}) at 30 °C:

\[
\rho_{oil} = \frac{m_{oil}}{m_{H2O}} \cdot \rho_{H2O}
\]

(1)

where \(m_{oil}\) is the mass of coconut oil (g) and \(m_{H2O}\) is mass of water (g), and \(\rho_{H2O}\) is the density of water (g/cm³). A LVT Synchro-lectric viscometer (Brookfield Engineering Laboratories, Stoughton, MA) with low viscosity spindle number LV1 was used at a speed of 30 rpm to measure the viscosity of coconut oil at 30 °C (n = 3). AOCS Official Method Cd 8-53- Peroxide value: acetic acid-chloroform method (16) was followed to confirm the oxidative stability of coconut oil after 28 days of storage at 30 °C.

**HPLC Analysis of Natural Antioxidants in Coconut Oil.** Analyses were performed on an Agilent 1100 Series LC (Agilent Technologies, Santa Clara, CA) with a micro degasser, quaternary pump, autosampler, thermostated column compartment, and diode array detector (DAD). A 4.6 × 50 mm, 1.8 µm Zorbax Eclipse XDB-C8 reversed-phase analytical column, equipped with a 4.6 × 12.5 mm, 5µm Zorbax Eclipse XDB-C8 guard column was used at 30 °C. External standards of free α-tocopherol and quercetin dihydrate in 75:25 isopropanol:methanol were run with each sampling time to quantify antioxidant content in samples of treated coconut oil. Coconut oil samples were diluted 1:10 in 75:25 isopropanol:methanol, transferred to amber glass vials, and held in the autosampler at a temperature of 30 °C until injection. The
wavelength range of 200–400 nm was detected by DAD and used for spectral analysis of sample peak purity.

(A) α-Tocopherol method. Diluted coconut oil samples exposed to LLDPE films loaded with α-tocopherol and α-tocopherol:β-CD complex were eluted in isocratic mode in a mobile phase of 96:4 methanol:water for 12 min. The flow rate was 1.0 mL/min, the injection volume was 10 µL, and the detection wavelength by DAD was 292 nm. The retention time (t_R) was 2.5 min, capacity factor (k) was 2.7, LOD was 0.05 µg/mL, and LOQ was 0.16 µg/mL. Coconut oil (10%) in the sample solvent was observed to have several small peaks at 292 nm that eluted with the last detectable peak having a t_R of 10.2 min. Therefore, a 12 min total run time was selected for the α-tocopherol method to completely elute the background components in coconut oil.

(B) Quercetin method. Diluted coconut oil samples exposed to LLDPE films loaded with quercetin and quercetin:γ-CD complex were eluted in isocratic mode in a mobile phase of 72:28 0.025 M KH_2PO_4 buffer, pH 2.4:acetonitrile for 4 min (modified from Hertog et al. (17)). The flow rate was 1.0 mL/min, the injection volume was 5 µL, and the detection wavelength by DAD was 372 nm. The retention time (t_R) was 2.6 min, capacity factor (k) was 2.4, LOD was 0.05 µg/mL, and LOQ was 0.17 µg/mL. No background components were observed at 372 nm in 10% coconut oil in the sample solvent.

The small injection volumes used above were optimized and free of solvent influence; however, larger injection volumes resulted in solvent influence on peak shape. Sensitivity of the DAD was optimized by the following parameters for both methods: 16 nm slit width, >0.2 min (4 s) peak width, and 16 nm bandwidth. ChemStation for LC 3D software (Agilent Technologies, Santa Clara, CA) was used for data management.

**Solvent Extraction of Natural Antioxidants.** Preliminary extractions were performed on 1.3-g metallocene LLDPE films (n = 1) with antioxidant additives placed in contact with 15.0 mL of four different organic solvents. Extraction was in two stages of one 24-hour exposure followed by an additional 48 hours. THF, acetonitrile, isopropanol, and n-hexanol were selected as test solvents based on their lower densities than LLDPE, good solvating power for α-tocopherol and quercetin, and range in solubility parameters. Solubility of α-tocopherol and
Quercetin in THF was confirmed by rapid solubilization of these antioxidants at a concentration level 10 times greater than the theoretical maximum loading in LLDPE.

LLDPE samples were cut into pieces of less than 10 mm in any dimension. These polymer samples were freeze-fractured in liquid N₂ and immediately transferred to the hopper of grinding equipment. A Mikro-Pulverizer (Pulverizing Machinery, Summit, NJ) grinder fitted with a stainless steel sieve of 3.175-mm diameter holes was used for size reduction. A distribution of approximately 1–2 mm and smaller LLDPE particles was collected. A three-stage extraction of 24 hours each in THF at 30.0 °C and 100 rpm was performed by replacing the extraction solvent with fresh THF after each 24-hour period. Particulate LLDPE samples (1.0 g) containing antioxidant additives were added to 20.0 mL of THF. After each 24-hour period, 1.0 mL of THF was diluted 1:10 in 75:25 isopropanol:methanol and analyzed by the same HPLC method as was used for coconut oil samples to quantify the amount of antioxidant in free and CD complexed forms within the LLDPE (n = 3).

Calculation of Solubility Parameter. The Hildebrand solubility parameter (δ) may be calculated from knowledge of the chemical structure of any compound and by using the group molar attraction constants (G) for each group as follows:

\[
\delta = \rho \sum \frac{G}{M}
\]

where \( \rho \) is the density and \( M \) is the molecular weight. Group molar attraction constants at 25 °C calculated by Small (18) were used for all groups, except –COOH group which used Konstam and Fearheller (18). The solubility parameter of coconut oil was calculated from the weighted mass fractions of the fatty acid composition reported by the supplier. Quercetin was calculated in its anhydrous form due to the thermal conditions experienced during polymer processing.

Estimation of Partition Coefficient. The Hildebrand solubility parameters (δ) of α-tocopherol, LLDPE, and coconut oil can be used to estimate the partition coefficient since this model system involves nonpolar solutes in nonpolar solvents. The partition coefficient (\( K_{FP} \)) for the distribution of additive, A, between food, F, and polymer, P, can be estimated as follows (19):
\[ \ln K_{FP} = \frac{(\delta_A - \delta_P)^2 V_A \phi_P^2}{RT} - \frac{(\delta_A - \delta_F)^2 V_A \phi_F^2}{RT} \]  

(3)

where \( \delta_A \) is the solubility parameter for additive, \( A \); \( \delta_P \) is the solubility parameter for the polymer, \( P \); \( \delta_F \) is the solubility parameter for food, \( F \); \( V_A \) is the molar volume of additive, \( A \); \( \phi_P \) is the volume fraction of polymer, \( P \); \( \phi_F \) is the volume fraction of food that is assumed to approach one; \( R \) is the gas law constant; and \( T \) is the absolute temperature. The volume fraction of polymer, \( P \), \((\phi_P)\) may be calculated as follows:

\[ \phi_P = \frac{V_p(1 - X_A^P)}{V_p(1 - X_A^P) + V_A X_A^P} \]  

(4)

where \( V_p \) is the molar volume of the polymer, \( P \), and \( X_A^P \) is the mole fraction concentration of additive in the polymer phase.

**Migration Model. Limited Packaging, Limited Food.** The mathematical solution for a normal Fickian diffusion process with fixed boundary conditions, as the additive migrates from a plane sheet into a stirred liquid, has been solved by Crank (20). The numerical evaluation of the solution generally is dependent upon series expansions and converges very slowly for early times or small amounts of additive migrating. The most rigorous general model for describing the migration controlled by Fickian diffusion in a packaging film is given by (21):

\[ \frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=1}^{\infty} \frac{2 \alpha(1 + \alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp \left[ -\frac{D q_n^2 t}{L_p^2} \right] \]  

(5)

where \( M_{F,t} \) (mg) is the mass of additive in the food at time \( t \); \( M_{F,\infty} \) (mg) is the mass of additive in the food at infinite time; \( \alpha \) is the mass ratio of additive in the food to that in the polymer film at equilibrium; \( q_n \) are the non-zero positive roots of \( \tan q_n = -\alpha q_n \); \( D \) is the diffusion coefficient \( (\text{cm}^2 \text{s}^{-1}) \); \( L_p \) is the half thickness of the polymer film (cm); \( t \) is time (s); and \( n \) is the index variable. The concentration mass ratio, \( \alpha \), was calculated using:

\[ \alpha = \frac{K_{FP} V_F}{V_p} \]  

(6)

where \( K_{FP} \) is the partition coefficient for the distribution of additive between food, \( F \), and polymer, \( P \); \( V_F \) is the volume of the food; and \( V_P \) is the volume of the polymer. Microsoft Excel was used to fit the model parameters of active concentration fraction \( (f) \), diffusion coefficient
(D), and concentration mass ratio (α) by minimizing the sum of squares error (Σε²). The Excel Solver tool was automated to determine the eigenvalues (q_i) for the estimated value of K_{FP}.

**Statistical Analysis.** Free α-tocopherol data were normalized since it was not possible to deliver the same weight of the viscous liquid to LLDPE resin with exact precision across three separate compounded batches. Statistical analysis of the registered variables (antioxidant additive, LLDPE resin type, and storage time) was performed by a split-plot design with a whole-plot factor in a generalized randomized block design with the general linear model supported by SAS (Version 9.1.3, 2003, SAS, Cary, NC). Multiple comparisons were adjusted for the Tukey-Kramer method of the general linear model procedure to test for least-squares mean separation of α-tocopherol concentration in coconut oil. Significant interactions were analyzed using the slicing function of the general linear model procedure. Effects were considered significant at p < 0.05.

α-Tocopherol concentrations released into 95% ethanol at a storage time of 28 days were analyzed by a two-way analysis of variance with the general linear model supported by SAS with the registered variables (antioxidant additive and LLDPE resin type). The same multiple comparison adjustment and alpha level as above were selected. Data values were reported as mean ± standard error.

**RESULTS AND DISCUSSION**

**HPLC Analysis of Natural Antioxidants in Coconut Oil.** Flavonoids, such as quercetin, are commonly analyzed by reversed-phase HPLC (17, 22, 23). The quantification of flavonoids enriched in cottonseed oil requires an extraction step to isolate the antioxidants from the lipid matrix followed by reversed-phase HPLC (24). α-Tocopherol can be analyzed by either normal-phase HPLC (25, 26) or it requires an extraction step followed by reversed-phase HPLC (27, 28). Elimination of an extraction step has proven to lead to more accurate quantification of phenolic antioxidants and tocopherols (29). In the current method, no further sample preparation is required besides dilution of the coconut oil in the sample solvent of 75:25 isopropanol: methanol at 30 °C. Removing the extraction step not only simplifies the analysis but also greatly reduces the time in which antioxidants can undergo oxidative degradation prior to injection. The reversed-phase HPLC method described in this study allows the quantification of α-tocopherol
and quercetin from coconut oil diluted into the same sample solvent and separated on the same C8 analytical column. Two separate mobile phases were required due to the extreme range in polarity of these two antioxidants as indicated by their solubility parameters in Table 5.1. The capacity factors (k) of both antioxidants were optimized between 2 and 5, which is the ideal range for isocratic separations.

**Natural Antioxidant Extractions from LLDPE.** The initial antioxidant concentration in LLDPE films was not able to be determined by dissolution of the films in toluene at 65 °C as had been reported previously for low density polyethylene (LDPE) (13, 30). Suitable solvents were, therefore, explored to extract the natural antioxidant additives from the LLDPE. THF was selected as the solvent with the highest extraction efficiency for α-tocopherol and quercetin within the LLDPE matrix (Table 5.4). Solubility parameters in Table 5.1 provide a quantitative basis for understanding why THF permeates and swells polyethylene, while the other tested solvents acetonitrile, isopropanol, and n-hexanol were not effective. Polymers will typically dissolve in solvents having solubility parameters within about two $\delta$MPa$^{1/2}$ units of their own (31). THF possess a higher solubility parameter value than the other solvents, thereby showing greater propensity to interact with the LLDPE chain segments. THF sorption and desorption curves for LLDPE at 25 °C are very fast relative to other organic penetrants and exhibit a maximum mass sorption of about 17% THF into LLDPE (32). Solvent extraction methods using THF have been reported previously to determine the initial α-tocopherol content within loaded LDPE films (33, 34).

In Table 5.2, α-tocopherol retention in Ziegler-Natta (ZN) and metallocene (met) LLDPE was calculated as 68 and 79%, respectively. Retention of quercetin in ZN and met LLDPE was calculated as 79 and 91%, respectively. CD complexes of both natural antioxidants in the two LLDPE resin types were found to have little free antioxidant extracted by THF. Extraction with organic solvent is one of the industrial methods of releasing guest molecules from CD inclusion complexes. THF has been used successfully for the release of various organic guests from inclusion complexes with α-, β-, and γ-CD (35). THF’s ability to dissociate the inclusion complexes of both α-tocopherol and quercetin from their host CD was confirmed. These CD complexes within the polymer matrix exhibit different dissociation behavior than in their free form as solid powders.
The THF extraction appeared complete in terms of the final stage not containing quantifiable amounts of the natural antioxidants. However, observations of quercetin in LLDPE support the concept that some active concentration fraction of antioxidant remains in the polymer after the extraction. ZN and met LLDPE films containing quercetin retained some degree of their characteristic yellow color even after 79% and 91% of their respective theoretical maximum concentration was extracted, which indicates quercetin or a colored degradation product remained bound in the LLDPE matrix. Additionally, a 100% release of \( \alpha \)-tocopherol from ZN LLDPE into coconut oil at 28 days of storage is calculated assuming that the THF extraction is complete. Its migration curve did not reach an equilibrium condition at 28 days, which indicates that a higher active concentration of \( \alpha \)-tocopherol remained available for release from the ZN LLDPE film. It was concluded that the natural antioxidant additives were not completely extracted from LLDPE by THF and, therefore, the antioxidant retention values in Table 5.2 do not serve as an accurate representation of the mass of antioxidant transferred to coconut oil at equilibrium (\( M_{F,\alpha} \)). The active concentration fractions (\( f \)) of the natural antioxidant additives were estimated from the migration model-fit procedure.

**Controlled Release of \( \alpha \)-Tocopherol from LLDPE.** *Coconut Oil.* Vegetable oils likely provide very similar mass transfer data to those occurring in real fatty food–packaging systems (36). The primary reason provided for the infrequent use of oils is the complication of the analysis due to numerous oil components and their lack of volatility. Triglyceride adsorption into LDPE is low (<2%, v/v) and was reported to have no effect on additive migration rate (37). The density and viscosity of the coconut oil model system at 30 °C was 0.915 g/cm\(^3\) and 34.5 ± 0.2 cps, respectively. Vegetable oils having lower viscosities and lower solidification points are known to extract higher concentrations of antioxidants and other additives from polymers (9). The level of endogenous \( \alpha \)-tocopherol in coconut oil was 5.7 ± 0.3 mg/L (n = 8), while quercetin was not present at detectable levels. Coconut oil samples were stable to oxidation after 28 days of storage at 30 °C as expected with peroxide values of <0.5 meq O\(_2\)/kg. The oxidative stability of this model food system ensured that neither \( \alpha \)-tocopherol nor quercetin was consumed when released from LLDPE films into coconut oil throughout the storage period.

Two different LLDPE resin types produced by Ziegler-Natta (ZN) and metallocene (met) catalysis were previously used to compound natural antioxidant additives and compression mold
them into films (14). These LLDPE films have the same density, but different crystalline structure morphologies and diffusion path networking arrangements that may allow for differences in antioxidant additive release rate. Conventional multi-site ZN LLDPEs are characterized by broad molecular weight distributions and broad comonomer distributions. Single-site catalyzed met LLDPEs are extremely uniform polymers with copolymers of narrow molecular weight distribution and narrow comonomer distribution. A narrower comonomer distribution results in a narrower crystallite size distribution in the crystalline morphology. This narrower crystalline size distribution will have a significant impact on additive diffusion and release rate through the material. Control of crystalline morphology translates into control of diffusion path tortuosity of additives.

Complexation of \(\alpha\)-tocopherol with \(\beta\)-CD allows for the sustained release of a low concentration of antioxidant which may prove of great significance in inhibiting oxidative reactions in foods. The rate of migration was expressed as the ratio of the migrated amount of \(\alpha\)-tocopherol at a specific time to that at equilibrium (\(M_{F,t}/M_{F,\infty}\)). In Figures 1 and 2, the migration rate of \(\alpha\)-tocopherol in its \(\beta\)-CD complex from both LLDPE films indicates that there is not a free \(\alpha\)-tocopherol component in the \(\beta\)-CD complex. If free \(\alpha\)-tocopherol was present in the CD complex additive, then a rapid initial release of \(\alpha\)-tocopherol would be expected at short times. In Figure 5.1, 83% \(\alpha\)-tocopherol was released from ZN LLDPE in its free form compared to 9% \(\alpha\)-tocopherol released in its CD inclusion complex at 28 days of storage at 30 °C. In Figure 5.2, 57% \(\alpha\)-tocopherol was released from met LLDPE in its free form compared to 7% \(\alpha\)-tocopherol released in its CD inclusion complex at 28 days of storage at 30 °C. The relatively broad molecular weight distribution of ZN LLDPE is known to increase overall molecular mobility, which is likely responsible for the greater release rate of \(\alpha\)-tocopherol compared to met LLDPE.

Antioxidant additive, LLDPE resin type, and storage time all had significant effects on the concentration of \(\alpha\)-tocopherol released from the polymer into coconut oil. Free \(\alpha\)-tocopherol release from ZN and met LLDPE became significantly different from initial time at days 1 and 3, respectively. \(\alpha\)-Tocopherol released from its \(\beta\)-CD complex in both LLDPE resin types did not differ significantly from initial time over the 28-day storage period. In ZN LLDPE films, the concentration of \(\alpha\)-tocopherol in its free and \(\beta\)-CD complex forms became significantly different beginning at day 1. In met LLDPE films, there was a significant difference in \(\alpha\)-tocopherol
concentrations between free and β-CD complex forms starting at day 3. Free α-tocopherol concentrations in ZN and met LLDPE films became significantly different beginning at day 7. There was no significant difference in concentrations of α-tocopherol complexed in β-CD between ZN and met LLDPE over the 28-day storage period.

95% Ethanol. Type of antioxidant additive had a significant effect on α-tocopherol concentration released into 95% ethanol. In Figure 5.3, free and β-CD complexed α-tocopherol additives were significantly different at 28 days of storage time in both ZN and met LLDPE. 95% Ethanol was used as a fatty food simulant (13) for relative comparison to coconut oil, however, ethanol cannot be considered realistic for specific migration of potential migrants. LLDPE resin type did not exhibit a significant difference in 95% ethanol, while a significant difference was present in coconut oil. In Figure 5.3, ZN and met LLDPE films containing α-tocopherol released 72% and 55%, respectively, of the antioxidant, which was similar to coconut oil at the 28-day time point. Ethanol does not appear to function effectively as a food simulant for α-tocopherol:β-CD complex loaded polymer films. The β-CD complex of α-tocopherol in ZN and met LLDPE films showed α-tocopherol release of 23% and 27%, respectively. This is more than twice the α-tocopherol concentrations released to coconut oil, which may be attributed to ethanol functioning more effectively at dissociating the β-CD complex within the LLDPE matrix.

Performance of Quercetin in LLDPE. Quercetin and its γ-CD complex were not released (<LOD) from the LLDPE films into coconut oil or 95% ethanol throughout the entire 28-day storage period. Quercetin was compounded into the polymer at levels above its intrinsic solubility and, therefore, existed as a saturated solution of quercetin in the polymer and a separate phase of particulate quercetin. Such additives are known to have low diffusion coefficients and minimal migration. This two-phase system was observed by optical microscopy in a previous study (14). The very low concentration of soluble quercetin was effective in the stabilization of LLDPE from oxidative degradation, but its limited solubility holds the majority of the quercetin in phase-separated domains within the polymer, thereby inhibiting release.
**Migration Model. Limited Packaging, Limited Food.** Migration is the sum of both diffusion and partitioning processes. The diffusion coefficient represents the additive migration rate and the partition coefficient represents the ratio of the additive concentration in the packaging to the additive concentration in the food at equilibrium. The partition coefficient ($K_{FP}$) for the distribution of $\alpha$-tocopherol between coconut oil and LLDPE was 1.8 as estimated from the procedure to calculate $K_{FP}$ from solubility parameter data. Holding the $K_{FP}$ value at 1.8 gave a concentration mass ratio ($\alpha$) of 19. In this case, the parameters of active concentration fraction ($f$) and diffusion coefficient ($D$) were fit to the model by reducing the sum of squares errors between the actual and model values. Table 5.3 summarizes the selected curve-fit parameters for the migration model.

Some amount of $\alpha$-tocopherol was presumably lost during the compounding and compression molding process, which resulted in an $f$ less than the theoretical value ($f = 1.0$). The presence of a commercial stabilizer system in the LLDPE likely reduced the tocopherol loss by competing with $\alpha$-tocopherol to capture radicals formed in the system during processing. The $f$ values for $\alpha$-tocopherol after polymer processing were estimated as 0.87 and 0.91 for ZN and met LLDPE, respectively, from the curve-fit procedure of the migration model. These estimated $f$ values are very comparable to other studies which processed 2000 mg/kg $\alpha$-tocopherol concentrations into LDPE (13, 30, 38). It is also appropriate that the $f$ in ZN LLDPE would be less than that of met LLDPE, since ZN LLDPE control films were found to have a lower degree of oxidative stability (14).

$\alpha$-Tocopherol in its $\beta$-CD inclusion complex was assumed to have at least equivalent thermal and oxidative stability as free $\alpha$-tocopherol since CD complexation typically provides increases in stability. The $\alpha$-tocopherol:$\beta$-CD complex also was found to function more effectively in maintaining the oxidative stability of LLDPE compared to free $\alpha$-tocopherol (14), which would indicate at least an equivalent $\alpha$-tocopherol level in its CD complex. $\alpha$-Tocopherol and its $\beta$-CD complex were, therefore, assigned the same $f$ in each LLDPE resin type. Any specific dissociation process of $\alpha$-tocopherol from its $\beta$-CD inclusion complex within the bulk LLDPE and its potential effect on $K$ was not considered in this model.

The selected mathematical model was robust and very efficient at fitting the experimental migration data to the modeled migration curve. The highest sum of squares error of $3.0 \times 10^{-3}$
was found to be one (13) and two (30) orders of magnitude less than the curve fit of a different model for two independent sets of experimental data of α-tocopherol migration from LDPE. The y-intercept of the modeled migration curve does cross at zero at short time (near \( t = 0 \)) due to the fact that a series approximation for the model was used which is best at long times. The accuracy of the model approximation at short time could be improved by adding more terms in the series or using an error function model for short times.

The diffusion coefficients calculated in this study are in agreement with the experimental values of various migrants as a function of molecular weight from LLDPE and LDPE (39). In ZN LLDPE, the calculated values for \( D \) of α-tocopherol and its β-CD complex were \( 4.2 \times 10^{-10} \) and \( 4.7 \times 10^{-12} \) cm²s⁻¹, respectively. In met LLDPE, the values for \( D \) of α-tocopherol and its β-CD complex were calculated as \( 2.0 \times 10^{-10} \) and \( 1.8 \times 10^{-12} \) cm²s⁻¹, respectively. The \( D \) of α-tocopherol in LLDPE decreases by a factor of about 100 in its complexed form with β-CD. This can be attributed to the large molecular weight increase during association of α-tocopherol and β-CD. The \( D \) of α-tocopherol in both free and β-CD complexed form decreases by a factor of 2 in met LLDPE compared to conventional ZN LLDPE. Met LLDPE has narrower crystalline size distributions in the crystalline morphology, which are able to decelerate diffusion of α-tocopherol through the bulk polymer. The control of crystalline morphology enables controlling diffusion path tortuosity of additives and their corresponding release rates from packaging films.

Polymer additive migration into a food product is dependent upon numerous factors including the original concentration of the additive in the polymer, its solubility in the food, its partition coefficient between the polymer and food, temperature, and time. Limited solubility of an additive in the polymer may not allow release due to phase segregation of the additive in the bulk polymer. Increasing the molecular weight of an additive by CD complexation can greatly reduce \( D \). Polymers with different crystalline structure morphologies and diffusion path networking arrangements allow for differences in additive release rates. Effective controlled-release packaging should combine CD complexation of additives and polymer morphology control to target delivery of an optimal antioxidant concentration to achieve prolonged activity, resulting in extended shelf life foods.
ABBREVIATIONS USED
CD, cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; LLDPE, linear low density polyethylene; LDPE, low density polyethylene; THF, tetrahydrofuran; ZN, Ziegler-Natta; met, metallocene; $f$, active concentration fraction; $\alpha$, concentration mass ratio; $D$, diffusion coefficient; $K$, partition coefficient

ACKNOWLEDGMENT
This research is based upon work supported by the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) of the National Science Foundation under Agreement No. DGE-0333378. The authors would like to thank Ronald D. Moffitt of the Advanced and Applied Polymer Processing Institute (AAPPI) for his assistance with the mathematical modeling of migration data.
LITERATURE CITED


### Table 5.1. Hildebrand Solubility Parameters ($\delta$) of Solvents (40), Polyethylene (18), Coconut Oil, and Natural Antioxidants at 25 °C

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\delta$ MPa$^{1/2}$</th>
<th>Material</th>
<th>$\delta$ MPa$^{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetonitrile</td>
<td>24.3</td>
<td>polyethylene</td>
<td>16.5</td>
</tr>
<tr>
<td>ethanol</td>
<td>26.0</td>
<td>coconut oil</td>
<td>18.5</td>
</tr>
<tr>
<td>n-hexanol</td>
<td>21.9</td>
<td>$\alpha$-tocopherol</td>
<td>18.3</td>
</tr>
<tr>
<td>isopropanol</td>
<td>23.5</td>
<td>quercetin</td>
<td>34.1</td>
</tr>
<tr>
<td>methanol</td>
<td>29.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tetrahydrofuran</td>
<td>18.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>47.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2. Extraction of Antioxidant Additives from LLDPE Films into Tetrahydrofuran

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>Antioxidant Concentration</th>
<th>Antioxidant Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st stage</td>
<td>2nd stage</td>
</tr>
<tr>
<td></td>
<td>mg/L</td>
<td>mg/L</td>
</tr>
<tr>
<td>Ziegler-Natta LLDPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>91.2 ± 0.2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>α-tocopherol:β-CD complex</td>
<td>18.9 ± 2.9</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>quercetin</td>
<td>70.1 ± 0.3</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>quercetin:γ-CD complex</td>
<td>3.7 ± 0.1</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>Metallocene LLDPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>103.0 ± 0.7</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>α-tocopherol:β-CD complex</td>
<td>23.7 ± 0.2</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>quercetin</td>
<td>81.2 ± 0.5</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>quercetin:γ-CD complex</td>
<td>36.2 ± 2.2</td>
<td>11.6 ± 0.8</td>
</tr>
</tbody>
</table>
Table 5.3. Curve-Fit Parameters of Active Concentration Fraction ($f$), Diffusion Coefficient ($D$), and Concentration Mass Ratio ($\alpha$) Selected to Minimize the Sum of Squares Error ($\Sigma \varepsilon^2$) of the Migration Model

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>Polymer Film</th>
<th>$f$</th>
<th>$D$</th>
<th>$\alpha$</th>
<th>$\Sigma \varepsilon^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-tocopherol</td>
<td>ZN LLDPE</td>
<td>0.87</td>
<td>4.2E-10</td>
<td>19</td>
<td>3.0E-03</td>
</tr>
<tr>
<td>$\alpha$-tocopherol:β-CD complex</td>
<td>ZN LLDPE</td>
<td>0.87</td>
<td>4.7E-12</td>
<td>19</td>
<td>2.1E-03</td>
</tr>
<tr>
<td>$\alpha$-tocopherol</td>
<td>met LLDPE</td>
<td>0.91</td>
<td>2.0E-10</td>
<td>19</td>
<td>1.8E-03</td>
</tr>
<tr>
<td>$\alpha$-tocopherol:β-CD complex</td>
<td>met LLDPE</td>
<td>0.91</td>
<td>1.8E-12</td>
<td>19</td>
<td>2.4E-03</td>
</tr>
</tbody>
</table>
Figure 5.1. Mass fraction of $\alpha$-tocopherol released from Ziegler-Natta LLDPE film into coconut oil during 4-week storage at 30 °C in its (a) free and (b) $\beta$-cyclodextrin complexed forms. Solid curves represent the migration model fitted to experimental data points.
Figure 5.2. Mass fraction of α-tocopherol released from metallocene LLDPE film into coconut oil during 4-week storage at 30 °C in its (a) free and (b) β-cyclodextrin complexed forms. Solid curves represent the migration model fitted to experimental data points.
Figure 5.3. Mass fraction of α-tocopherol released from Ziegler-Natta and metallocene LLDPE into 95% ethanol at 4 weeks of storage at 30 °C in its free and β-cyclodextrin complexed forms.
Table 5.4. Various Solvent Extraction Efficiencies of Antioxidant Additives from Metallocene LLDPE Films (45.2-mm diameter and 870-μm thickness)

<table>
<thead>
<tr>
<th>Antioxidant Additive</th>
<th>% of Theoretical Extracted by Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>91.2</td>
</tr>
<tr>
<td>α-tocopherol:β-CD complex</td>
<td>16.2</td>
</tr>
<tr>
<td>quercetin dihydrate</td>
<td>100.0</td>
</tr>
<tr>
<td>quercetin:γ-CD complex</td>
<td>27.6</td>
</tr>
</tbody>
</table>
Figure 5.4. HPLC chromatograms of standards of (a) α-tocopherol (10 mg/L) and (b) quercetin dihydrate (8 mg/L) in sample solvent of 75:25 isopropanol:methanol.
Figure 5.5. HPLC chromatograms of α-tocopherol released from Ziegler-Natta LLDPE films containing the additives (a) free α-tocopherol and (b) α-tocopherol:β-CD complex into coconut oil at 28 days of storage at 30.0 °C.
ABSTRACT

The effectiveness of controlled-release antioxidant packaging films in inhibiting the formation of primary and secondary oxidation products in a tocopherol-stripped corn oil model system was monitored. However, an unstabilized, oxidatively susceptible food substrate with a low peroxide value was not available from any commercial supplier. \(\alpha\)-Tocopherol-loaded LLDPE films behaved as prooxidants during storage from a high initial peroxide value of 20.6 meq O\(_2\)/kg. At 28 days of storage, Ziegler-Natta and metallocene LLDPE films had peroxide values of 44.8 and 43.9 meq O\(_2\)/kg, respectively, compared to their respective control films with peroxide values of 40.0 and 40.8 meq O\(_2\)/kg. An SPME/GC-MS method to monitor the secondary oxidation products in corn oil is proposed. The DVB/CAR/PDMS fiber resulted in the best detection of pentane, hexanal, and \textit{trans}-2-heptenal produced from linoleic acid oxidation in corn oil.

KEYWORDS
cyclodextrin, inclusion complex, natural antioxidant, \(\alpha\)-tocopherol, quercetin, corn oil, oxidation, linear low density polyethylene, active packaging
INTRODUCTION

One of the most serious problems with the protection of shelf-stable foods is oxidation due to its deteriorating effects on food quality. Lipid oxidation may diminish food quality by decreasing nutritional quality, increasing toxicity, the development of off-odor, and altering texture and color (1). Lipid peroxidation may proceed even in the presence of very low concentrations of initiating free radicals due to its radical chain mechanism. It is therefore particularly important to intercept these initiators early with antioxidants. The use of active packaging to attain a controlled release rate of antioxidants would be ideal to inhibit the oxidative reaction directly on the food surface.

This study aims to show the prooxidant effect of α-tocopherol when added in one initial dose compared with its antioxidant activity at low concentrations released from polymer packaging films. α-Tocopherol provides its greatest antioxidant activity at lower concentrations and either decreases or exhibits prooxidant activity at higher concentrations in bulk vegetable oils. The optimal concentration for α-tocopherol to exhibit greatest antioxidant potency is approximately 100 ppm in corn (2), soybean (3, 4), and fish (5) oils. Most of the phenolic acids, flavonoids, anthocyanidins, and anthocyanins exhibit some prooxidant activity at very low concentrations (6). The prooxidant effect of flavonoids is most pronounced at very low antioxidant concentrations (<1–2 µM), and is reversed at higher concentrations (7).

Cyclodextrin (CD) complexes of the natural antioxidants, α-tocopherol and quercetin, have been prepared previously and incorporated into linear low density polyethylene (LLDPE) films of two different catalyst types in an earlier study (8). Quercetin and its γ-CD complex were not released from the LLDPE films into coconut oil throughout a 28-day storage period. The release kinetics of α-tocopherol in its free and β-CD inclusion complexed forms from LLDPE films into a coconut oil model system have been estimated with a migration model. The purpose of this research was to monitor the effectiveness of a controlled-release antioxidant packaging film in inhibiting the formation of primary and secondary oxidation products in a tocopherol-stripped corn oil model system. The controlled release of natural antioxidants from polymer packaging may more effectively limit oxidation, maintain nutritional quality, inhibit the formation of harmful oxidation products, and extend the shelf life of foods.
MATERIALS AND METHODS

Materials. (+)-α-Tocopherol of 98% purity and quercetin dihydrate of 99% purity were supplied by Sigma-Aldrich (St. Louis, MO). Pentane (99.5%), hexanal (99.2%), and trans-2-heptenal (97.6%) were purchased from Sigma-Aldrich (St. Louis, MO). A refined-grade of stripped corn oil was supplied by Acros Organics (Geel, Belgium) with specifications of 0.918 g/cm³ density, <25 µg/g total tocopherols, and a peroxide value of <5 meq O₂/kg. Ziegler-Natta and (ZN) metallocene (met) LLDPE films loaded with 2715 mg/kg α-tocopherol in its free and β-CD complexed form, and 1950 mg/kg quercetin in its free and γ-CD complexed form were prepared as described previously (8). Chloroform and isooctane of HPLC grade, and glacial acetic acid of ACS reagent grade were obtained from Fisher Scientific (Pittsburgh, PA).

Stripped Corn Oil Model System. Clear glass straight-sided jars (60 mL) with polytetrafluoroethylene faced polyethylene-lined caps (Fisher Scientific, Pittsburgh, PA) were used as migration test cells with an inner diameter of 48.8 mm and a total volume of 74.5 mL. LLDPE sample films with 45.2 mm diameter and weight of 1.30 ± 0.005 g were submerged in 15.0 mL stripped corn oil in each test cell and placed in a PsycroTherm (model G-26) controlled environment incubator shaker (New Brunswick Scientific, Edison, NJ) at 30.0 ± 0.5 °C and 100 rpm protected from light. An air headspace volume of 58.1 mL was present in each test cell. Samples were removed for analysis of primary (peroxide value and conjugated dienes) and secondary oxidation products (SPME/GC-MS) at 0, 1, 3, 7, 14, 21, and 28 days of storage time. Only one replication was performed since stripped corn oil with a low peroxide value was not able to be obtained from any commercial supplier.

Viscosity of Corn Oil. A stripped corn oil sample was equilibrated at 30 °C in a water bath. A Synchro-lectric viscometer model LVT (Brookfield Engineering Laboratories, Stoughton, MA) with low viscosity spindle number LV1 was used at a speed of 30 rpm to measure corn oil viscosity (n = 3).
**Peroxide Value and Conjugated Diene Acid.** AOCs Official Methods Cd 8-53 Peroxide value: acetic acid-chloroform method (9) and Ti 1a-64 Spectrophotometric determination of conjugated dienoic acid (10) were used to measure primary oxidation products in bulk stripped corn oil (11).

**SPME/GC-MS.** Extraction and concentration of volatile secondary oxidation products in stripped corn oil was performed by solid-phase microextraction (SPME). A 2-cm 50/30 µm divinylbenzene/Carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) coated SPME fiber (Supelco, Bellefonte, PA) was exposed to the headspace above 5 mL of corn oil sample in a 10-mL headspace vial for 30 min at 40 °C with an agitation speed of 250 rpm. CTC Analytics Combi PAL system (Leap Technologies, Carrboro, NC) headspace injection was used for automation of sampling. Volatile compounds were desorbed for 2 min in the injector port of a gas chromatograph-mass spectrometer (GC-MS) system consisting of a HP 5890A GC gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a HP 5972 Series mass selective detector. The injector temperature was 250 °C, and all injections were made in the splitless mode. Separation was completed on a 30 m × 0.25 mm i.d. × 0.25-µm film thickness capillary column (HP-5MS; Hewlett-Packard Co., Palo Alto, CA) with a helium carrier gas flow rate of 1.1 mL/min (linear velocity of 30.5 cm/sec). The GC oven temperature was programmed at 35 °C for a 2-min hold time, increased to 120 °C at a rate of 5 °C/min, and ramped from 120 °C to 220 °C at a rate of 20 °C/min and held at 220 °C for 5 min. The MS was maintained at 240 °C and sample mass was scanned in the range of 40–200 amu. Gas chromatography-mass spectrometry (GC-MS) was performed to identify volatile secondary oxidation products in corn oil samples. Analyte retention times, tR (min) of pentane, hexanal, and trans-2-heptenal were 1.67, 5.62, and 10.54, respectively. Analyte mixed standards were prepared in the same stripped corn oil matrix as sample treatments for analysis by SPME/GC-MS with each sampling time point. Instruments were controlled with ChemStation B.02.05 (Hewlett-Packard Co., Palo Alto, CA) and MS chromatograms were analyzed using Enhanced ChemStation version B.01.00.

**Natural Antioxidants in Stripped Corn Oil.** α-Tocopherol (100 µM) and quercetin dihydrate (100 µM) were added to stripped corn oil which was equivalent to 43.1 mg/L and 33.8 mg/L, respectively. Solutions of α-tocopherol (100 and 200 mg/L) and fine suspensions of
quercetin dihydrate (100 and 200 mg/L) in stripped corn oil were prepared to determine oxidation induction time (OIT) using differential scanning calorimetry (DSC). Very fine dispersions of 100- and 200-mg/L quercetin in corn oil were prepared by 20 min of ultrasonication followed by 24 hours of shaking at 250 rpm and 25 °C.

**Oxidation Induction Time.** Stripped corn oil (5.5 ± 0.1 mg) which contained different concentrations of α-tocopherol and quercetin were tested for oxidative stability. Corn oil samples containing natural antioxidant additives were placed in an open standard aluminum DSC pan. A DSC Q1000 instrument (TA Instruments, New Castle, DE) was used with a N₂ sample purge flow of 50 mL/min. The DSC was equilibrated at 50 °C, ramped to 150 °C at 10 °C/min, and held isothermal for 5 min. The gas was then switched to O₂ holding the samples isothermally at 150 °C until stopping the run after the maximum slope of the exotherm was observed in the real-time DSC curve. Universal Analysis 2000 (TA Instruments, New Castle, DE) was used to analyze the OIT by the tangent method.

**RESULTS AND DISCUSSION**

**Stripped Corn Oil Model System.** Corn oil typically contains α-tocopherol at concentrations in the range of 112–143 mg/kg (12, 13) and γ-tocopherol at a level of 602 mg/kg (12). Tocopherol-stripped (<25 µg/g total tocopherols) corn oil was received with a peroxide value greater than the manufacturer specification (<5 meq O₂/kg) on numerous occasions. Stripped corn oil was still tested as a model system even though the material was moderately oxidized upon receipt from the supplier. Table 6.1 illustrates that the peroxide value of each of ten 400-g bottles of corn oil varied considerably between two different shipments of the same product lot number. At 30 °C, the viscosity of stripped corn oil was 38.9 ± 0.3 cps, which was very similar to the reported viscosity of coconut oil at 34.5 ± 0.2 cps used in natural antioxidant migration studies from LLDPE films (8).

Stripped corn oil, which contains about 57% linoleic acid (18:2ω6), serves as an easily oxidizable substrate for a model food system. Figure 6.1 shows the structures and main ion fragments of secondary oxidation products of linoleic acid including pentane, hexanal, and trans-2-heptenal. An unoxidized food system as indicated by low initial peroxide value that is also unstabilized is necessary to test the effectiveness of controlled-release antioxidant packaging
films in inhibiting the formation of primary and secondary oxidation products. The antioxidant effectiveness of phenolic compounds in virgin olive oils has been reported to be significantly diminished in oils if their initial peroxide values are too high ([14]). The first replication in this study used stripped corn oil with a peroxide value of 20.6 meq O$_2$/kg directly as received from the supplier.

Future tocopherol-reduced corn oil model systems should be prepared with closely monitored tolerances for research purposes with proper protection from light, heat, and oxygen during shipping. Evans et al. ([4]) presents a method for preparation of tocopherol-reduced soybean oil by short-path vacuum distillation (SPVD). This SPVD method resulted in removal of about 99% of total tocopherols without significantly altering the free fatty acid content, conjugated diene concentration, refraction index, fatty acid distribution, or the amount of *trans* fatty acids.

**Peroxide Value and Conjugated Dienoic Acid.** Primary oxidation products in stripped corn oil were monitored during storage in contact with LLDPE films loaded with natural antioxidant additives. From the previous migration study ([8]), it was shown that quercetin and its $\gamma$-CD complex were not released from LLDPE films into coconut oil, therefore, no antioxidant effect would be expected from these films. Ziegler-Natta (ZN) and metallocene (met) LLDPE control films had peroxide values of 40.0 and 40.8 meq O$_2$/kg, respectively, at 28 days of storage. In Figures 6.2 and 6.3, $\alpha$-tocopherol-loaded LLDPE films behave as prooxidants, on the basis of peroxide value. At 28 days of storage, ZN and met LLDPE films had peroxide values of 44.8 and 43.9 meq O$_2$/kg, respectively. At 28 days in a coconut oil model system, $\alpha$-tocopherol-loaded ZN and met LLDPE films released 169.8 ± 7.6 and 121.4 ± 10.9 mg/L, respectively ([8]). It is likely that very similar concentrations were released into the corn oil model system. The addition of 210 mg/L $\alpha$-tocopherol to corn oil in one initial dose resulted in an even greater prooxidative effect as indicated by a peroxide value of 50.6 meq O$_2$/kg at 28 days of storage. Corn oil with a starting peroxide value of 20.6 meq O$_2$/kg contains a high level of hydroperoxides. At high concentrations, $\alpha$-tocopherol inhibits hydroperoxide decomposition but promotes hydroperoxide formation ([15]). The addition of 150 mg/L quercetin in one initial dose resulted in a peroxide value of 39.6 meq O$_2$/kg at 28 days, which was at a level similar to control LLDPE. The conjugated dienoic acid method appeared to have less sensitivity to detect
treatment differences compared to the peroxide value iodometric titration method as shown in Figures 6.4 and 6.5.

**SPME/GC-MS.** The use of SPME to extract volatile compounds of lipid foods has only been reported in the past decade most likely due to the high solubility of these volatiles in vegetable oils. Pentane, hexanal, and \( \text{trans}-2\)-heptenal were the secondary oxidation products of linoleic acid from corn oil detected in this study. Steenson et al. (16) reported the polydimethylsiloxane (PDMS) solid phase had the second highest sensitivity to hexanal and the best coefficient of variation of the four phases tested in corn and soybean oils. Doleschall et al. (17) also compared SPME fibers for measuring volatile compounds in vegetable oils. They found the 2-cm 50/30 \( \mu \text{m} \) DVB/CAR/PDMS fiber was most appropriate in determination of volatiles from sunflower oil. The DVB/Carboxen/PDMS fiber was found to have much greater SPME extraction efficiency than the PDMS fiber for numerous volatile compounds in vegetable oil by several other researchers (18-20).

Method development testing of PDMS, Carboxen/PDMS, and DVB/CAR/PDMS fibers in this laboratory concluded that the DVB/CAR/PDMS fiber provided the best detection of pentane, hexanal, and \( \text{trans}-2\)-heptenal in corn oil (Figure 6.6). This laboratory is also in agreement with several other researchers (17-20) that the sensitivity of the PDMS fiber is very poor for the detection of volatiles in vegetable oils. The first replication of monitoring secondary oxidation products of stripped corn oil when stored in contact with different natural antioxidant treatments in LLDPE was not successful due to (a) the stripped corn oil having an initial peroxide value of 20.6 meq O\(_2/kg\), and (b) the preparation of volatile standards of pentane, hexanal, \( \text{trans}-2\)-heptenal in corn oil with the same lot number but a different degree of oxidation for the corn oil sample treatments. Figure 6.7 shows a GC-MS chromatogram of stripped corn oil (peroxide value of 20.6 meq O\(_2/kg\)) at initial time. Relatively large pentane and hexanal peaks are already present indicating the oil matrix is in the latter stages of oxidation.

**Oxidation Induction Time.** Accelerated testing for the determination of lipid oxidation may use high temperatures, but such results typically show poor correlation to oxidation rates under normal conditions. A comparative study successfully determined the oxidative stability of various edible oils by DSC (21). High-temperature oxidation stability data can only be
extrapolated to room temperature when there is no change in the participation mechanism of the antioxidant and its radical in the inhibited oxidation reactions (22). Increases in temperature from 25 to 100 °C have been shown to affect the mechanism of action of \( \alpha \)-tocopherol.

The observed exotherm of the OIT tests at 150 °C are indicative of the oxidative reactions in vegetable oil which have a similar profile to OIT determination of polyethylene. In Table 6.2, quercetin dihydrate exhibited a prooxidant effect at low concentrations of 100 \( \mu \)M as evidenced by a 3.5 min decrease in OIT from the control corn oil. \( \alpha \)-Tocopherol did not provide the unstabilized corn oil with any real protection from oxidation relative to the control until a slight antioxidant effect at the 200 mg/L level. Quercetin prooxidant activity increases with increasing concentration until the antioxidant activity of the compound rapidly becomes dominant at about 200 \( \mu \)M (7). Quercetin has been reported to be a more effective additive in reducing the oxidation level than \( \alpha \)-tocopherol at 200 ppm in a bulk stripped corn oil system (23). In bulk corn oil, the reduced oxidative protection of \( \alpha \)-tocopherol may be due to its homogeneous distribution in solution in the bulk lipid phase rather than orienting at the oil–air interface (24). More effective protection could be attained at this interface, where hydrophilic antioxidants orient themselves.

The reported solubility limit of quercetin in soybean oil is approximately 100 \( \mu \)M (33.8 mg/L) (25). The antioxidant activity of quercetin has been shown to be twice that of \( \alpha \)-tocopherol at a 5 mM concentration determined by oxidative stability index values (26). This study is in agreement that the insolubility of flavonoids in oil does not prevent them from acting as antioxidants (26). Increasing inhibition of fish oil oxidation was observed with increasing concentrations of quercetin in excess of its solubility from 300–1000 mg/kg (27). Frankel et al. (24) also observed that ascorbic acid in suspension in bulk corn oil is particularly active. This remarkable activity of partially soluble suspended ascorbic acid may be attributed to its more favorable orientation at the air–oil interface where surface oxidation occurs. Low solubility with an effective antioxidant is not a disadvantage if the diffusion or dissolution rates are not determining factors (28).

Evaluation of oxidative stability ideally should be performed at temperatures as low as possible dependent upon the oxidative susceptibility of the oil (14). Different volatile compounds have been reported to form dependent upon the temperature of the oxidation study (29). In the current and previous studies, the activity of natural antioxidants was studied at 30 °C.
because it is very close to conditions under which oxidation protection of edible oils is required. This temperature also allowed the saturated coconut oil to exist as a liquid for migration experiments without the risk of solidification.

An unstabilized, oxidatively susceptible food substrate with a low peroxide value was not able to be obtained from any commercial supplier. Nature has engineered its own oxidative protection mechanism by providing many of the more unsaturated vegetable oils with a higher level of endogenous tocopherols. Short-path vacuum distillation should be used as an effective method to obtain a tocopherol-stripped corn oil as a model food system that contains a low level of hydroperoxides. Therefore, future controlled-release packaging studies will be able to measure an effect in the initial phases of the oxidative radical chain reaction with much greater sensitivity.

**ABBREVIATIONS USED**

CD, cyclodextrin; β-CD, β-cyclodextrin; γ-CD, γ-cyclodextrin; SPME, solid-phase microextraction; DVB, divinylbenzene; CAR, Carboxen; PDMS, polydimethylsiloxane; DSC, differential scanning calorimetry; SPVD, short-path vacuum distillation
LITERATURE CITED

1. Finley, J. W.; Given, P., Jr. Technological necessity of antioxidants in the food industry. Food and Chemical Toxicology 1986, 24, (10/11), 999-1006.


### Table 6.1. Peroxide Values of Ten Stripped Corn Oil Bottles Received in Three Different Shipments (A, B, and C)

<table>
<thead>
<tr>
<th>Shipment</th>
<th>Date</th>
<th>Corn Oil, Stripped</th>
<th>Peroxide Value (meq O₂/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>10/9/2007</td>
<td>Lot A018245701</td>
<td>6.6</td>
</tr>
<tr>
<td>A2</td>
<td>10/9/2007</td>
<td>Lot A018245701</td>
<td>7.2</td>
</tr>
<tr>
<td>A3</td>
<td>10/9/2007</td>
<td>Lot A018245701</td>
<td>7.0</td>
</tr>
<tr>
<td>A4</td>
<td>10/9/2007</td>
<td>Lot A018245701</td>
<td>4.2</td>
</tr>
<tr>
<td>B1</td>
<td>11/14/2007</td>
<td>Lot A0247016</td>
<td>1.4</td>
</tr>
<tr>
<td>B2</td>
<td>11/14/2007</td>
<td>Lot A0247016</td>
<td>2.0</td>
</tr>
<tr>
<td>B3</td>
<td>11/14/2007</td>
<td>Lot A0247016</td>
<td>1.8</td>
</tr>
<tr>
<td>C1</td>
<td>11/15/2007</td>
<td>Lot A0247016</td>
<td>7.0</td>
</tr>
<tr>
<td>C2</td>
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<td>Lot A0247016</td>
<td>6.4</td>
</tr>
<tr>
<td>C3</td>
<td>11/15/2007</td>
<td>Lot A0247016</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Table 6.2. Oxidation Induction Time (OIT) of Natural Antioxidants in Stripped Corn Oil at 150 °C

<table>
<thead>
<tr>
<th>Natural Antioxidant</th>
<th>Concentration (mg/L)</th>
<th>OIT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.0</td>
<td>19.1</td>
</tr>
<tr>
<td>(\alpha)-tocopherol</td>
<td>43.1*</td>
<td>19.0</td>
</tr>
<tr>
<td>(\alpha)-tocopherol</td>
<td>100</td>
<td>19.6</td>
</tr>
<tr>
<td>(\alpha)-tocopherol</td>
<td>200</td>
<td>17.9</td>
</tr>
<tr>
<td>quercetin dihydrate</td>
<td>33.8*</td>
<td>15.6</td>
</tr>
<tr>
<td>quercetin dihydrate</td>
<td>100</td>
<td>20.4</td>
</tr>
<tr>
<td>quercetin dihydrate</td>
<td>200</td>
<td>22.9</td>
</tr>
</tbody>
</table>

* Equivalent to a concentration of 100 µM
Figure 6.1. (a) Pentane, (b) hexanal, and (c) trans-2-heptenal are secondary oxidation products of linoleic acid, a primary component of corn oil.
Figure 6.2. Peroxide value of stripped corn oil with different natural antioxidant treatments in Ziegler-Natta LLDPE during 4 weeks of storage at 30 °C.
Figure 6.3. Peroxide value of stripped corn oil with different natural antioxidant treatments in metallocene LLDPE during 4 weeks of storage at 30 °C.
Figure 6.4. Conjugated dienoic acid of stripped corn oil with different natural antioxidant treatments in Ziegler-Natta LLDPE during 4 weeks of storage at 30 °C.
Figure 6.5. Conjugated dienoic acid of stripped corn oil with different natural antioxidant treatments in metallocene LLDPE during 4 weeks of storage at 30 °C.
Figure 6.6. SPME/GC-MS chromatogram of mixed standards of pentane (26.0 mg/kg), hexanal (10.1 mg/kg), and *trans*-2-heptenal (2.1 mg/kg) spiked into a stripped corn oil matrix.
Figure 6.7. SPME/ GC-MS chromatogram of pentane, hexanal, and trans-2-heptenal in stripped corn oil (peroxide value of 20.6 meq O₂/kg) at initial time.