EFFECT OF CONTROLLED RELEASE OF TOCOPHEROL ON LIPID OXIDATION

by

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ABSTRACT OF THE THESIS

Effect of Controlled Release of Tocopherol on Lipid Oxidation

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Lipid oxidation is one of the major problems affecting the shelf life of fatty foods. Controlled release packaging (CRP) is an innovative packaging technology that incorporates antioxidants into packaging materials and releases them in a controlled manner to food products thus providing continuous replenishment of antioxidants to food. The objective of this research is to study the release behaviors of tocopherol (antioxidant) in CRP films and simulate these release behaviors with different initial concentrations to examine the effect of controlled release of tocopherol on lipid oxidation.

Low density polyethylene (LDPE), polypropylene (PP) and LDPE/PP blend films were produced by coextrusion process to contain 3000 ppm of tocopherol. Release behaviors of tocopherol in the package films were studied and diffusivity values of tocopherol in above three films were estimated from the release study at different temperatures, which ranged from 2.04×10^{-16} m^2/s to 2.39×10^{-13} m^2/s. Release profiles of tocopherol from LDPE and LDPE/PP films were simulated in a syringe pump using

estimated diffusivities and two initial tocopherol concentrations (100 and 300 ppm) to inhibit lipid oxidation of linoleic acid at 40 °C. The induction period of lipid oxidation was determined to evaluate the antioxidant effectiveness of tocopherol with different release profiles. Induction period changed significantly with controlled release compared to instant addition. For releasing of 100 ppm tocopherol, release by D_{LDPE}, the induction period was 73 hours, longer than that of instant addition (57 hours). For releasing by $D_{LDPE/PP}$, the induction period was 47 hours, shorter than instant addition. For releasing of 300 ppm tocopherol, release by D_{LDPE}, the induction period (213 hours) was twice of that from instant addition (91 hours).

This work demonstrated that controlled release of tocopherol from CRP films has great impact on induction period extension of lipid oxidation. Less tocopherol can be applied to achieve longer induction period compare to instant addition. However, too slow diffusion of tocopherol in certain polymer may not have the effect to prevent lipid oxidation. This work provides partial quantitative information to help researchers and packaging manufacturers select suitable initial concentration and polymer types based on required shelf life.

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1. INTRODUCTION

Packaging provides different functions for food products: containment, protection, convenience and communication [1]. Besides the basic function as a container, many efforts have been done on packaging development to protect foods from physical force, environmental change and food degradation. Meanwhile, shelf life is a critical issue for food products, which is usually terminated by food degradation or severe environmental conditions.

New packaging technologies utilize the packaging functions to prevent food degradation and extend shelf life. One of these value-added packaging technologies is named controlled release packaging (CRP). CRP is such a new generation of packaging technology that incorporates active compounds (antioxidants or antimicrobials) into packaging films, and then releases them in a desired mode from package to food to extend shelf life and enhance food quality. Compared with traditional preservation way in which additives are incorporated into initial food formulation and consumed in a short period, CRP can provide continuous replenishment of antioxidants or antimicrobials to food by controlled releasing them from package, thus preventing lipid oxidation or minimizing microbial spoilage to extend the shelf life [2].

Using CRP to prevent lipid oxidation for fatty food products is the focus for this study – antioxidant CRP. Lipid oxidation is the main chemical reaction resulting in food degradation and shelf life termination for fatty food products [3]. There are three stages

during lipid oxidation: initiation, propagation and branching, and termination [4]. Since the reaction speeds up after the initiation stage immediately and the reaction rate increases exponentially, antioxidant usually functions in initiation stage, which is also called induction period, an indicator for shelf life [5]. Antioxidants are used to quench free radicals and stop the reaction chains during induction period, thus extending the induction period as long as possible to reach required shelf life. Different synthetic antioxidants have been used already in food industry to prevent lipid oxidation, e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG). However, health concerns lead people to prefer natural antioxidants to synthetic ones [6, 7].

Tocopherol is concerned to be a promising candidate as a natural antioxidant for CRP, which is well known for its effective inhibition of lipid oxidation in foods [8]. It was first introduced into packaging area due to its excellent ability as a stabilizer during polyolefins processing [9-13]. Research showed that tocopherol in the films still retains antioxidant effect after extrusion process under severe conditions [14, 15]. Therefore, the idea of antioxidant CRP came out: more amount of tocopherol could be applied into films as an antioxidant, and then be controlled released from package to food to retard lipid oxidation.

However, how to determine initial tocopherol concentration incorporated into films and polymer choice? It refers to release of tocopherol from CRP films to food, which is critical for controlling food degradation reactions. Too slow release of tocopherol may not provide sufficient amount of tocopherol to prevent oxidation, while too fast release may

cause pro-oxidation [16]. Before controlling the release of tocopherol to match food degradation, release behaviors of tocopherol in different polymers need to be studied and quantitative information are necessary for the design of CRP to achieve appropriate release. Three steps are involved in the release of tocopherol: diffusion in the packaging polymer, dissolution at package/food interface and diffusion in the food. Compared to dissolution and diffusion in the food, diffusion of tocopherol in the polymer is a rate determine step during the whole release process in most cases. It mainly depends on the temperature, tocopherol molecular mass, and its chemical affinity with the polymer [1]. It provides quantitative information about how fast tocopherol can move in different polymers. This diffusion information can link with food degradation rate, and then researchers can choose optimal polymer for different food products to extend the shelf life.

As to initial concentration of tocopherol loaded into the CRP films, it is not practical to produce all the films with different concentrations. Therefore, a syringe pump is introduced to simulate the release behaviors of tocopherol from real CRP films with different initial concentrations to examine the effect on lipid oxidation of linoleic acid.

Although timed-release of tocopherol already showed the effect of induction period extension on lipid oxidation compared to instant addition [17], from a practical point of view, the effect of controlled release of tocopherol using release behavior from real CRP films need to be studied. Antioxidant CRP is still a new area needed more investigation to study the effect of controlled release of antioxidant on lipid oxidation.

2. LITERATURE REVIEW

2.1. Lipid Oxidation Reaction

Lipid oxidation has been concerned as a major problem affecting the shelf life of fatty foods. It results in deterioration of flavor, color and texture [18], degradation of nutritional and food safety qualities [19], even negative effects on carcinogenesis, premature aging and other diseases [20].

Chemically, lipid oxidation is a process that unsaturated fatty acids in phospholipids and triacylglycerides react with oxygen and degrade to a variety of volatile and nonvolatile products. It is a dynamic process with no fixed intermediates or endpoints. The reactions change with time and conditions presents distinct challenges in measuring and controlling lipid oxidation [4]. Generally speaking, lipid oxidation is a free radical chain reaction which includes three stages: initiation, propagation and branching, and termination (Figure 1). In the initiation stage, free radical $L^\bullet$ is formed and catalyzed by catalysts, e.g. light, metals and heat. Then $L^\bullet$ reacts with oxygen to form peroxyl radicals, $LOO^\bullet$, which is a major player in propagation stage. Hydroperoxides and new $L^\bullet$ are formed by $LOO^\bullet$ and the reaction is repeated again and kept going. Chain propagation continues until no hydrogen source is available or the chain is intercepted. Radical chain reaction is expanded and faster in the second stage of propagation, called branching. L_nOOH forms $LO^\bullet$, which dominates in the reactions and is a faster chain carrier. Termination is the closing stage of lipid oxidation although lipid oxidation never stops

fully, because radical always leaves behind another radical to continue the chain reaction.

Initiation: $LH \longrightarrow L^{\bullet}$

Propagation: $L_1^{\bullet} + O_2 \longrightarrow L_1OO^{\bullet}$

$L_1OO^{\bullet} + L_2H \longrightarrow L_1OOH + L_2^{\bullet} \longrightarrow L_2OO^{\bullet}$

$L_2OO^{\bullet} + L_3H \longrightarrow L_2OOH + L_3^{\bullet}$ etc. $\cdots\cdots\rightarrow L_nOOH$

& Branching: $L_nOOH \Big\rangle \quad L_nO^{\bullet} + OH^{-}$

$L_nOOH \longrightarrow L_nOO^{\bullet} + H^{+} \Big\rangle \longrightarrow$ more chain reactions

$L_nOOH \Big\rangle \quad L_nO^{\bullet} + {}^{\bullet}OH$
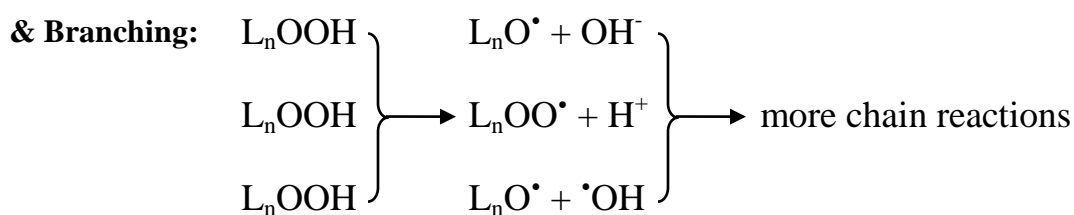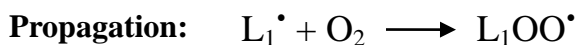
Termination:
a. Radical recombinations:

Alkyl radicals $(L_1^{\bullet} + L_2^{\bullet}) \longrightarrow$ alkane polymers
Peroxyl radicals $(L_1OO^{\bullet} + L_2OO^{\bullet}) \longrightarrow$ alcohols and ketones, alkyl peroxides
Alkoxyl radicals $(R_1O^{\bullet} + R_2^{\bullet}) \longrightarrow$ ethers, ketones, alkanes
$(R_1O^{\bullet} + R_2 O^{\bullet}) \longrightarrow$ alkyl peroxides, ketones, alcohols

b. Scission Reactions of $LO^{\bullet}$:

$$\overset{\beta}{R_1}\overset{\alpha}{\underset{O^{\bullet}}{CH}}\!R_2\!-\!COOH \longrightarrow R_1^{\bullet} + \overset{\beta}{\underset{O}{CH}}\!-\!R_2\!-\!COOH \quad \text{or} \quad \overset{\alpha}{R_1}\underset{O}{CH} + {}^{\bullet}R_2\!-\!COOH$$

c. Co-oxidation of Nonlipid Molecules:

$$LH \longrightarrow L^{\bullet} \overset{O_2}{\longrightarrow} LOO^{\bullet} \overset{}{\not\longrightarrow} LOOH + L^{\bullet} \text{ etc}$$

$\Big\downarrow$ Protein

$$Protein^{\bullet} \longrightarrow Protein\text{-}OO^{\bullet} \overset{O_2}{\longrightarrow} Protein\text{-}OOH$$
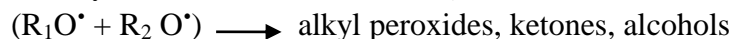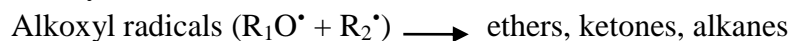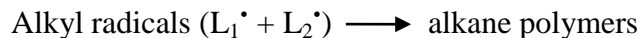
Figure 1: Three stages of lipid oxidation (Adopted from Schaich [4])

Three mechanisms terminate lipid free radicals to form nonradical products: (a) radical recombinations; (b) scission reactions when proton sources; (c) co-oxidation of nonlipid molecules such as proteins [4].

2.2. Shelf Life Determination for Fatty Foods

Shelf life has been defined as the period of time during which a food retains acceptable characteristics of flavor, color, aroma, texture, nutritional value, and safety under defined environmental conditions [1]. The major deterioration mode determining the shelf life in fatty food is lipid oxidation: volatile odorous compounds change aroma, hydroxy acids cause flavor modifications, and formation of dimers and polymers increases viscosity, etc [19, 21]. All of these quality factors including flavor, texture, and lipid oxidation products have been used to determine the shelf life of food product under certain critical limits. Critical limit is the minimum value for quality factors based on consumers' acceptability as shown in Figure 2. For example, if hexanal, the volatile compound generated from lipid oxidation, exceeds the sensible threshold, consumers can smell the rancid odor and will then reject the food [3].
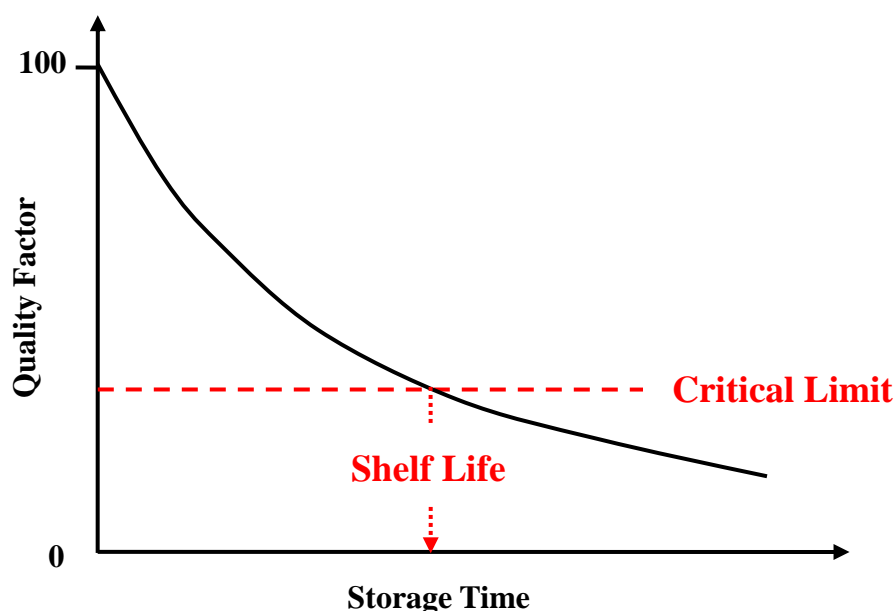
Figure 2: Shelf life determination by quality factor under critical limit

As to analytical measurement for quality of fat or oil, different procedures are used to measure peroxide value, volatile organic compounds and so on. One common method is measuring the concentration of conjugated dienes, which is generated during oxidation due to double bonds change from nonconjugated to conjugated bonds [21]. Figure 3 demonstrates the integration and progression of individual reactions of initiation, propagation, and termination into an overall oxidation process. As shown in Figure 3, conjugated dienes is the primary product of lipid oxidation. In the early stage of lipid oxidation, it accumulates at a very stable and low level. As the reaction goes by, it increases exponentially that indicates oxidation is taking off, and rancidity could be detected. Thus, the induction period is defined as the length of time before detectable rancidity, or time before rapid acceleration of lipid oxidation [21]. Usually, it is used as

an indicator for shelf life. The longer induction period represents longer shelf life.
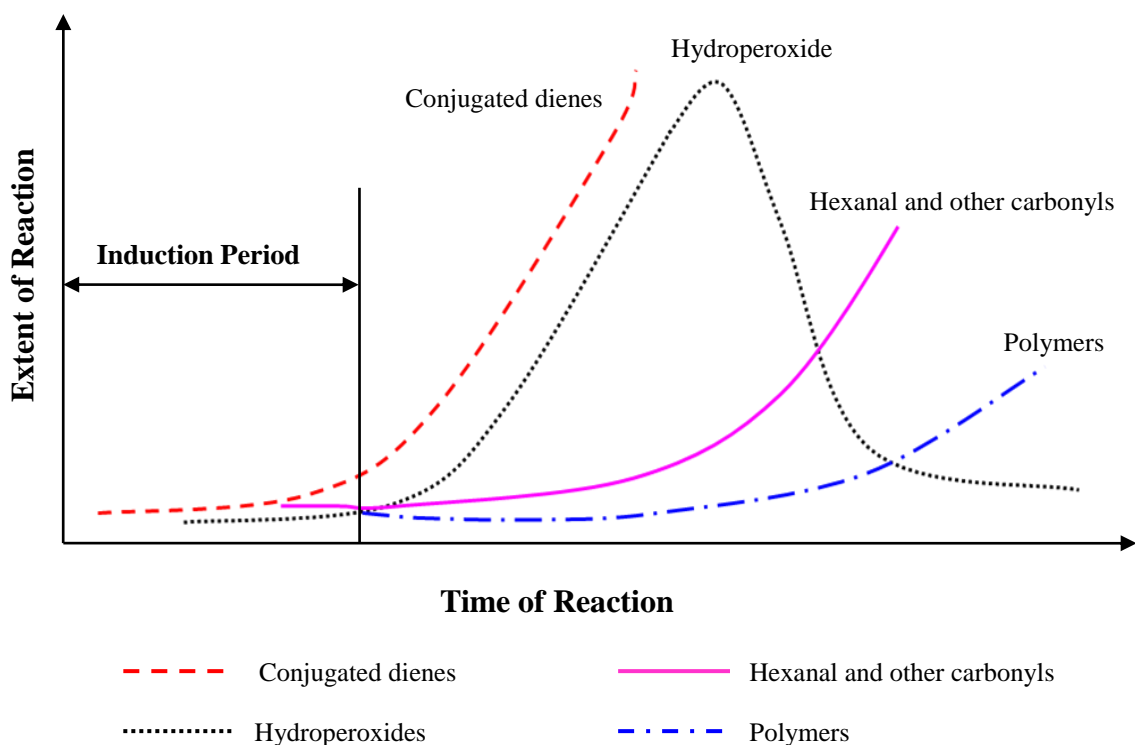
Figure 3: Changes in dominant lipid oxidation reactions and products over the course of lipid oxidation (Adopted from Schaich [4])

In this work, conjugated dienes were measured over time. Linear regression was applied on data points after lipid oxidation taking off. The calculated x intercept was induction period in Zhu's dissertation [17]. However, induction period was modified into the intersection of the rapid lipid oxidation line and initial conjugated dienes value at time zero (Figure 4).
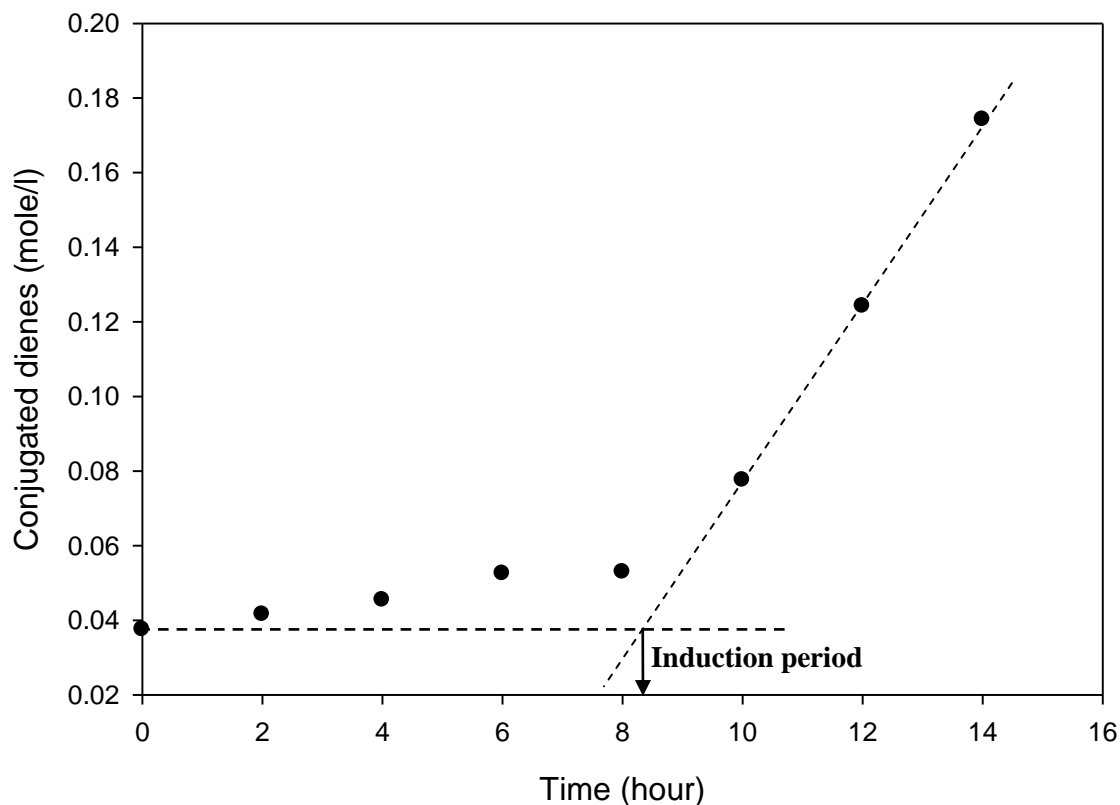
Figure 4: Determine the induction period of lipid oxidation by linear regression (Adopted from Zhu [17])

2.3. Antioxidant—Tocopherol

Antioxidants are commonly used to inhibit lipid oxidation, thus achieving long shelf life. Tocopherols are non-toxic compounds with a positive public perception, broad regulatory approvals, and environmentally friendly appeal to the consumer [2]. It is well known for its effective inhibition of lipid oxidation in foods and biological systems, e.g. pork patties [22], ground beef [23], peanut oil [24] and so on. It is a natural antioxidant present in oil seeds, leaves, and other green parts of higher plants. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT),

tert-butylhydroquinone (TBHQ) and propyl gallate (PG) are widely used in food industry. Compared with these synthetic antioxidants, tocopherols have excellent antioxidant properties (250 times that of BHT) mainly due to the heterocyclic ring in the chroman moiety [25]. There are mainly four homologues of tocopherol (Figure 5): α-tocopherol is present mainly in the chloroplasts of plant cells, while β-, γ-, and δ-homologues are usually found outside these organelles [26].
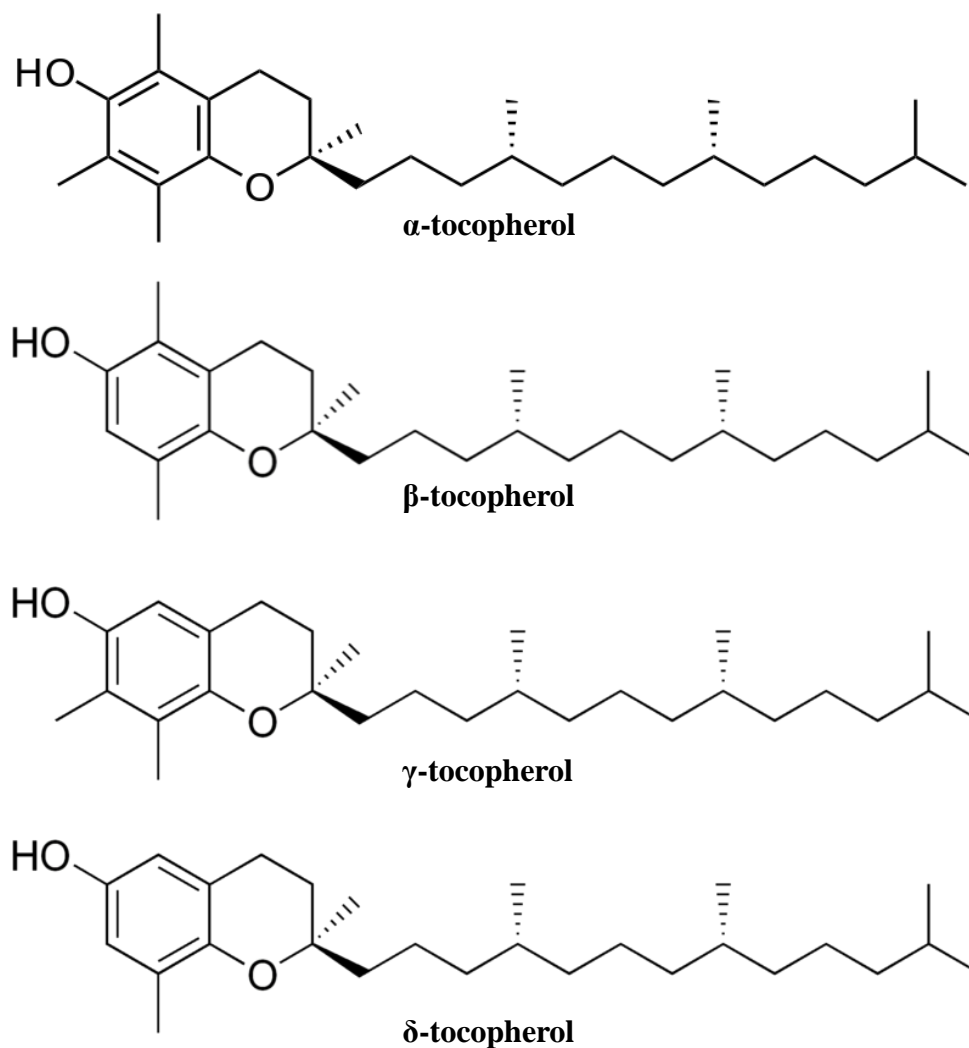
Figure 5: Molecular structures for different types of tocopherol

Their antioxidant activities generally depend on the ability to donate their phenolic hydrogens to lipid free radicals [25, 27, 28], which followed the order of α > β > γ > δ [27], and the relative antioxidant activity of the tocopherols *in vivo* is in this order [29-31]. However, the order is reversed in fats, oils and lipoproteins *in vitro*, which is δ > γ ≈ β > α [32-36]. The reasons may contribute to temperature, light, solvent or other chemical species acting as prooxidants and synergists in the system [8]. The tocopherol product used in this research is extracted from soybean that contains 10% α-, 5% β-, 65% γ-, and 20% δ-tocopherol homologues (Table 1).

Table 1: Composition in the natural tocopherol product used in this study

Homologues	Percentage
α-tocopherol	10%
β-tocopherol	5%
γ-tocopherol	65%
δ-tocopherol	20%

The mechanism of inhibition on lipid oxidation by tocopherol is shown in Figure 6. Tocopherol molecules (TOH) can quench the peroxy radicals (LOO$^{\bullet}$) to form tocopherol radicals (TO$^{\bullet}$), which are much less reactive and more stable to lipid radicals. Tocopherol can also react with alkoxy radicals (LO$^{\bullet}$) formed in the propagation step. In some case, when oxygen is only trace amounts and hydroperoxides are negligible concentrations, the tocopherols can react directly with alkyl radicals (L$^{\bullet}$). Tocopherol radicals can form tocopherol dimers which are more stable [8, 37]. Thus, tocopherol acts as a scavenger to

free radicals formed during lipid oxidation and stops the chain reactions finally.

$$LOO^{\bullet} + TOH \longrightarrow TO^{\bullet} + LOOH$$

$$LO^{\bullet} + TOH \longrightarrow TO^{\bullet} + LOH$$

$$L^{\bullet} + TOH \longrightarrow TO^{\bullet} + LH$$

$$TO^{\bullet} + TO^{\bullet} \longrightarrow TO\text{-}TO$$
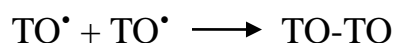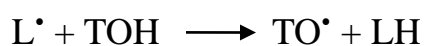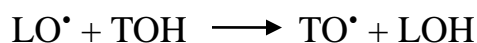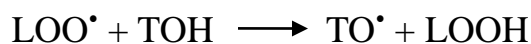
Figure 6: Mechanism of tocopherol on lipid oxidation inhibition (Adopted from Kamal-Eldin [8])

Tocopherol was first introduced into packaging area due to its excellent ability as a stabilizer during polyolefins processing [9-13]. Research showed that tocopherol in the films still has antioxidant effect under severe conditions during extrusion process [14, 15]. That opens another door to tocopherol that packaging films containing certain amount of tocopherol may provide antioxidant effect to food during storage by releasing tocopherol from package to food. Moreover, tocopherol has good solubility in lipid which means that there is driving force from food to release tocopherol from package to food.

2.4.   Controlled Release Packaging

Controlled release packaging (CRP) is still a new area where researchers have not fully studied. Controlled release of drug delivery has been used in pharmaceuticals for some period [38-41], and many interests are raised on controlled release of antioxidants or antimicrobials delivery by packaging to extend shelf life currently [42-46]. However, it has not been raised to quantitative level.

2.4.1.   Definition of Controlled Release Packaging

Controlled release packaging is a new packaging technology that incorporates antioxidants or antimicrobials into the package and then releases them in a controlled manner from package to food to enhance food quality and extend shelf life [2]. Traditionally, active compounds such as antimicrobials, antioxidants, and anti-browning agents are incorporated into initial food formulations; however, once these additives are consumed in reaction, protection ceases and food quality degradation increases rapidly. CRP can overcome this limitation by continuously replenishing active compounds via controlled release from packaging, which is necessary for achieving long term stability of foods [47].

Figure 7 demonstrate the mechanism of controlled release packaging: barrier layer is the outside of the whole controlled release packaging to protect food and prevent active compound (especially for volatile compounds) escape to outside, following is the active layer containing active compounds, and food are packed inside. During the storage, active compounds release from active layer into food at the desired rates, which match the degradation of the particular food.

Controlled Release Packaging
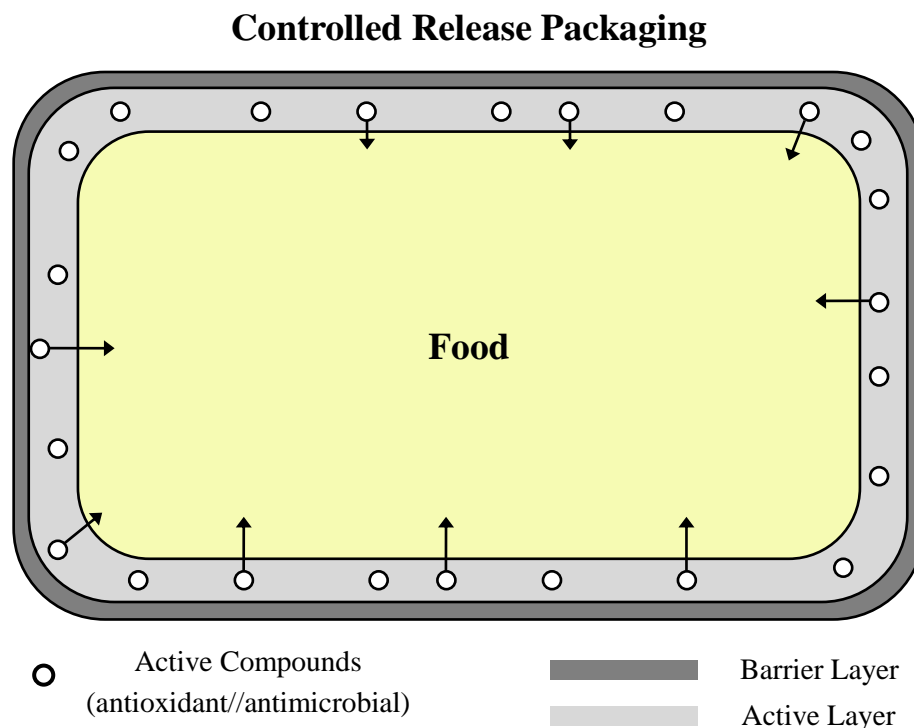
Figure 7: Illustration of controlled release packaging

The advantages of CRP are: (1) less active compounds are needed to incorporate into CRP compared to the one loaded into initial food formulation; (2) for fresh produce, it solves the problem that cannot add antimicrobial directly into food; (3) for long-term shelf life required food, the goal is achievable using CRP, e.g. three years shelf life for military meal ready to eat (MRE).

2.4.2.   Development of Packaging Systems

A systematical strategy was developed for CRP by Dr. Kit L. Yam and Dr. Karen M. Schaich as shown in Figure 8 [47]. The conceptual framework organizes the variables important to controlled release packaging into four groups: process variables, structure

variables, property variables, and food variables. It also divides the research activities into two major parts, packaging research and food research. It provides a research roadmap to elucidate the relationships between the variables necessary for advancing the development of controlled release packaging.

This study focuses on different process variables, including the concentration of active compound and polymer composition using extrusion, to bring different film structures, provide different release properties of active compound, and then influence the food degradation.
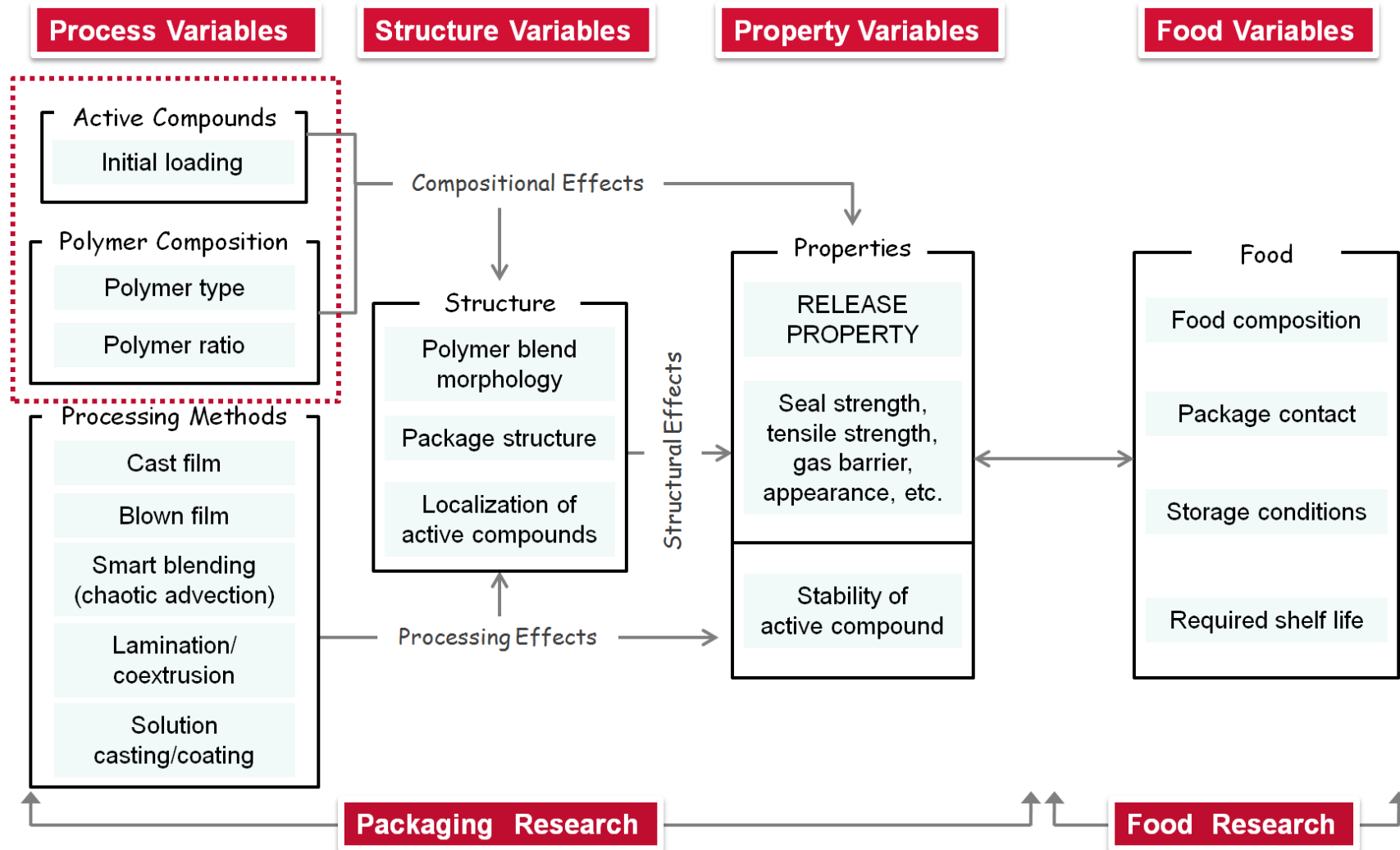
Figure 8: Conceptual framework for developing CRP (Adopted from Yam [47])

2.4.3.   Production of Controlled Release Packaging Film

Extrusion process is used in this research to produce tocopherol containing CRP films. The basic mechanism of extrusion is using heat and shear force to melt polymer resin, and forming a sheet or other forms though the specific die. As shown in Figure 9, polymer resins and tocopherol are well mixed and poured into the feeder. Screws in the barrel provide shearing to melt the resins along with high temperature, and force the polymer to merge into a feed block. A slit die attached to the feed block gives the sheet form to the film. Then, the polymer melt is drawn around chill rolls and cooled down to form a film. The whole roll system gives the orientation to the film. Adjust the rolling speed can give different film thicknesses and surface morphologies.
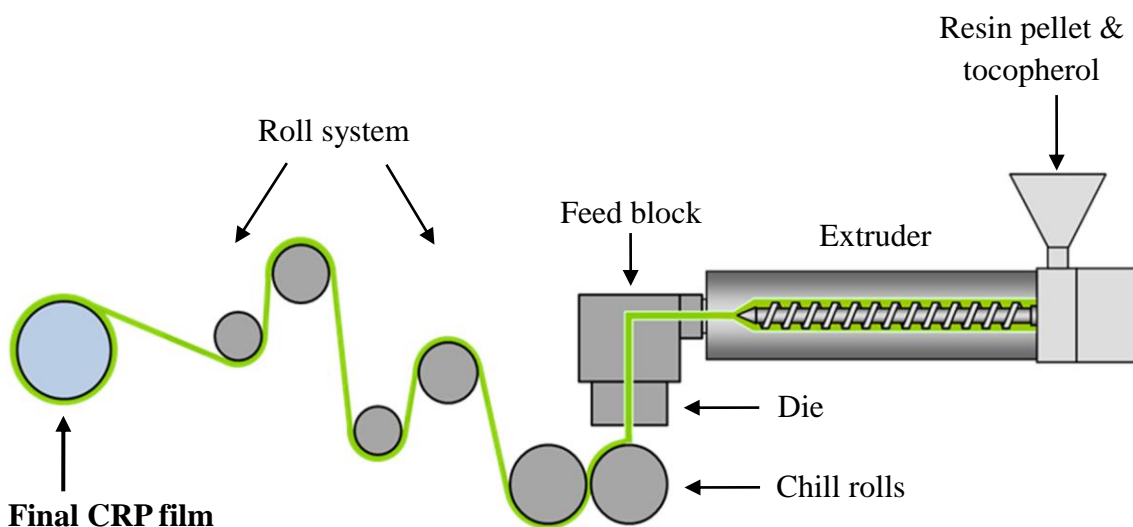
Figure 9: Extrusion production line to produce CRP films

The reasons of using extrusion to produce CRP films are: (1) keeping the consistence with previous work; (2) producing polymer films with more uniform film properties by commercial extrusion method; (3) enhancing the mixing of polymer melt and tocopherol, and the uniform distribution of tocopherol inside the polymer matrix by high shear generated by screw of extruder; (4) being easy to scale up in the future commercial production of tocopherol containing film since the extrusion is the major commercial film production method [17].

2.4.4.   Mechanism of Release

Three steps are involved in the release progress of active compounds from packaging to food (Figure 10): (1) diffusion in the polymer matrix; (2) dissolution at package/food interface; (3) diffusion in the food [48]. Sometimes, there's no diffusion of active compounds occurred in the food, since the active compounds only effect on the surface of the food.

Compared to dissolution and diffusion in the food, diffusion of tocopherol in the polymer is a rate determine step during the whole release process mostly. It mainly depends on the temperature, tocopherol molecular mass, and its chemical affinity with the polymer [1].

Since the driving force for the diffusion of active compounds in the polymer matrix is concentration gradient, the mass transport phenomena could be expressed by Fick's Law of diffusion [49]:

$$F = -D\frac{dC}{dx} \quad \text{(Equation 1)}$$

where F is the rate of transfer per unit area (moles/s), D is the diffusion coefficient, C is the concentration of diffusing substances (moles/m^3), and x is the distance diffused (m).

Package   **Food**

❶: Diffusion in the packaging film
❷: Dissolution at package/food interface
❸: Diffusion in the food
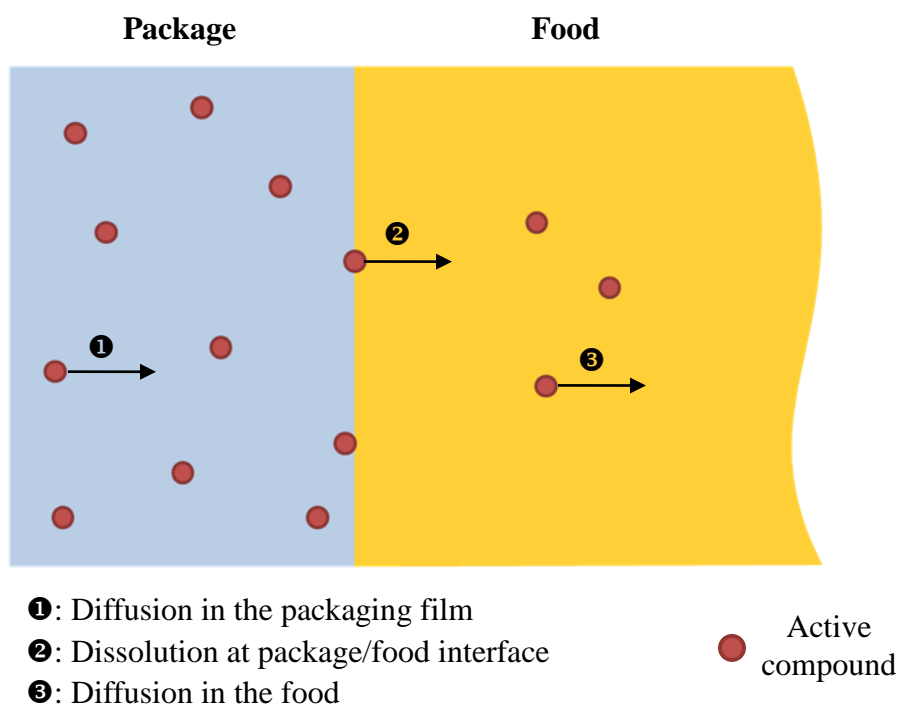
Active compound

Figure 10: Release mechanism of active compound from package to food

3. OBJECTIVE

3.1. Objective

The overall objective is to study the release behaviors of tocopherol in real CRP films and simulate these release behaviors with different initial concentrations to examine the effect of controlled release of tocopherol on lipid oxidation.

The overall objective can be mainly divided into three sub-objectives: (1) to obtain release behaviors of tocopherol in different CRP films; (2) to simulate tocopherol release from CRP films using different initial concentrations; (3) to examine the antioxidant effect of controlled release of tocopherol on lipid oxidation.

The impact of this research is that the delivery modes of tocopherol into linoleic acid are from the release profiles of tocopherol in real CRP films. Thus, the antioxidant effect of tocopherol on linoleic acid is close to the situation in a real package. From a practical point of view, the results can provide useful information to packaging designers to select suitable polymer and quantify the optimal concentration of tocopherol incorporated into the polymer for required shelf life.

3.2. Specific Tasks

- Controlled release packaging films production

- Total extraction of tocopherol from CRP films

- Release study of CRP films containing 3000 ppm tocopherol using 95% ethanol as

food simulant at different temperatures

- Matlab^{TM} programming for tocopherol diffusivity estimation in different polymers

- Simulation on tocopherol release profiles from CRP films with different initial concentrations

- Syringe pump programming using simulated data

- Lipid oxidation experiment of linoleic acid with different tocopherol delivery modes using syringe pump system

4. EXPERIMENTAL DESIGN

4.1. Experimental Design Overview

The experimental design is based on research objective written in the previous section. Figure 11 is the flow chart of the experimental design. It can be divided into three parts:

- Release kinetics study. It includes producing CRP films, following by total extraction to evaluate tocopherol retention in the films, release study to determine the release behaviors of tocopherol from films, and diffusivity estimation of tocopherol in the films to provide qualitative parameters for tocopherol release simulation.

- Syringe pump system simulation on tocopherol release profile. It includes simulating the release profiles of tocopherol from CRP films with different initial concentrations, and programming syringe pump using simulated data to mimic the release behaviors of tocopherol from real CRP films.

- Reaction kinetics study. It includes delivering tocopherol into linoleic acid using syringe pump system to examine the induction period of linoleic acid under different delivery modes.
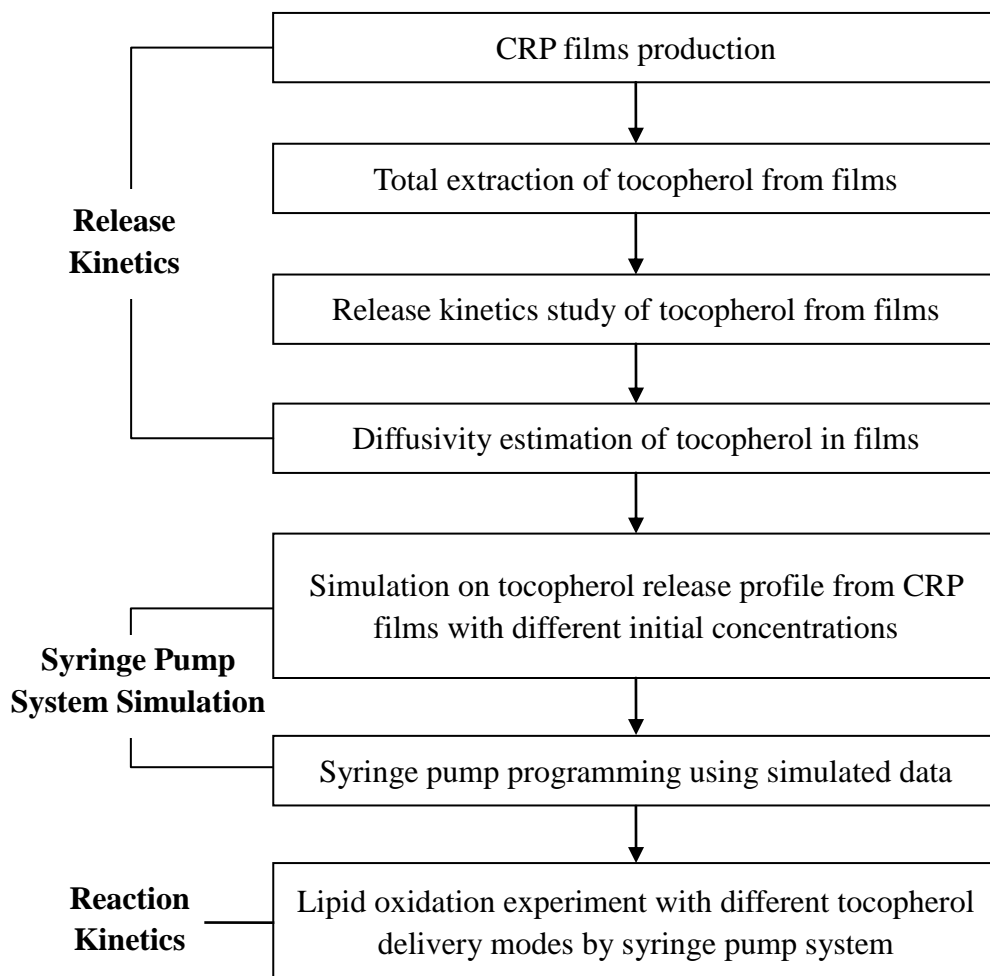
Figure 11: Experimental design flow chart

4.2. Materials

A natural tocopherol product extracted from soy bean containing α, β, γ, and δ-tocopherol was donated by Cargill Inc. (Eddyville, IA, USA). Polymer resins (resin without added antioxidant) low density polyethylene (LDPE), polypropylene (PP) were provided by Berry Plastics (Chippewa Falls, WI, USA). Linoleic acid (60%) and all organic solvents (HPLC grade) were purchased from Fisher Scientific Inc. (Suwanee, GA, USA).

4.3. Food Model System

In this study, two food model systems were used: (1) 95% ethanol was used in release study experiment as fatty food simulant to determine release kinetics of tocopherol from polymers at different temperatures, according to FDA 2007 guidelines for release studies from food-contact packaging materials [50]; (2) 60% linoleic acid was used in lipid oxidation experiment as food model to examine the antioxidant effect of tocopherol under different release modes.

4.4. Controlled Release Packaging Films Production

Three different CRP films containing tocopherol were designed to produce. Coextrusion was used for production in Berry Plastics (Chippewa Falls, WI, USA). Figure 12 is the overview of film production line. Due to the minimal requirement of production line, 14 lb polymer resins were mixed with 0.042 lb tocopherol to produce CRP films containing 3000 ppm tocopherol. LDPE, PP and 50/50% blend of LDPE/PP films were designed for tocopherol containing CRP films, and compositions of each film were listed in Table 2.

Figure 12: Overview of the film production line

Table 2: Experimental design of three tocopherol containing CRP films

Concentration of tocopherol (ppm)	Tocopherol amount in films (lb)	Polymer amount (lb)	
		LDPE	PP
3000	0.042	14	0
3000	0.042	7	7
3000	0.042	0	14

Thicknesses for three different tocopherol films were measured using Digitrix II Micrometers (Fowler NSK, Japan). Twenty points were selected randomly for each film. The average value was used as the thickness of the film.

4.5.   Total Extraction of Tocopherol from Films

Total extraction study was conducted to determine the retention of tocopherol in the films. Extractable tocopherol percentage was equal to the amount of extracted tocopherol divided by the amount of tocopherol originally added into films, which was showed in the following equation:

$$Extractable\ tocopherol\ (\%) = \frac{Extracted\ tocopherol}{Tocopherol\ originally\ added\ into\ films} \times 100$$

Shake-flask extraction method was used in total extraction study. 1 g film was cut into small pieces (1 cm × 1 cm) to maximize the surface area and placed into a 125 ml Pyrex flask. 40 ml methylene chloride was used to loss the film structure and extract tocopherol from the films. The flasks were rotary-shaken at 100 rpm in dark at 30 ℃ controlled by an environmental chamber (Lab-Line Instruments, Inc., IL, USA). The methylene chloride extracted solution samples were withdrawn after 48 hours, filtered with 0.2 μm PTFE filter (Millipore Corporation, MA, USA) and measured by UV/Vis spectrophotometer (UV1700, Shimadzu, Japan) at 295 nm. The concentration of tocopherol was determined by using a standard curve of tocopherol in methylene chloride.

4.6.   Release Kinetics Study of Tocopherol from Films

Release study experiment was conducted to determine release kinetics of tocopherol from different films. Released tocopherol percentage at a given time was equal to the amount of dissolved tocopherol in 95% ethanol divided by the amount of total extractable

tocopherol from films, which was showed in the following equation:

$$Released\ tocopherol\ (\%) = \frac{Disolved\ tocopherol\ in\ 95\%\ ethanol}{Total\ extractable\ tocopherol\ from\ films} \times 100$$

1 g film was cut into small pieces (1 cm × 1 cm), put into a 125 ml Pyrex flask piece by piece to prevent sticking, and immersed into 40 ml 95% ethanol in a 125 ml Pyrex flask. The flasks were rotary-shaken at 100 rpm in dark at 30, 40 and 50 ℃ controlled by the environmental chamber. The liquid samples were withdrawn periodically, filtered with 0.2 μm PTFE filter and measured by UV/Vis spectrophotometer at 295 nm. The concentration of tocopherol was determined by using a standard curve of tocopherol in 95% ethanol.

4.7.   Estimation of Tocopherol Diffusivity

Diffusion is the transport process from one part of a system to another as a result of random molecular motion. Diffusivity is the parameter of diffusion (also called diffusion coefficient, m^2s^{-1}). Diffusivity of tocopherol for unsteady state is determined by the analytical solution of Fick's Law in two dimensions for large migration times [49]:

$$\frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} exp\left[\frac{-D(2n+1)^2\pi^2 t}{L_P^{\ 2}}\right] \qquad \text{(Equation 2)}$$

where $M_{F,t}$ is the mass of the migrant in the food at a particular time t (s), $M_{F,\infty}$ is the mass of the migrant in the food at infinite time (in equilibrium), L_P (m) is the thickness of the film, D (m^2/s) is the diffusion coefficient of the migrant in the polymer, and t (s) is the

time. The following conditions were assumed: (1) mass transfer resistance of tocopherol from the film surface into the food simulant was negligible; (2) initial concentration of tocopherol in the food simulant was zero; (3) there was no concentration gradient of tocopherol in the food simulant; (4) partition coefficient and diffusion coefficient were constant at a given temperature; (5) interactions between the food simulant and the film were not considered. Diffusivities of tocopherol in different films at various temperatures were estimated from the results of release study using Matlab^{TM}. It was determined by minimizing the sum of the squares of errors (SSE) between the experimental and estimated values.

4.8.   Syringe Pump System Simulation

Syringe pump system (Figure 13) is used to simulate the release behaviors of tocopherol from different CRP films. It is controlled from a microcontroller based system which drives a step motor, allowing a large range of pumping rates configured to the inside diameter of the loaded syringe. The syringe is driven from a drive-screw and drive-nut mechanism. It can be programmed in the computer (Figure 14) to control the flow rate during infusing.

Figure 13: Syringe pump with its controlling computer
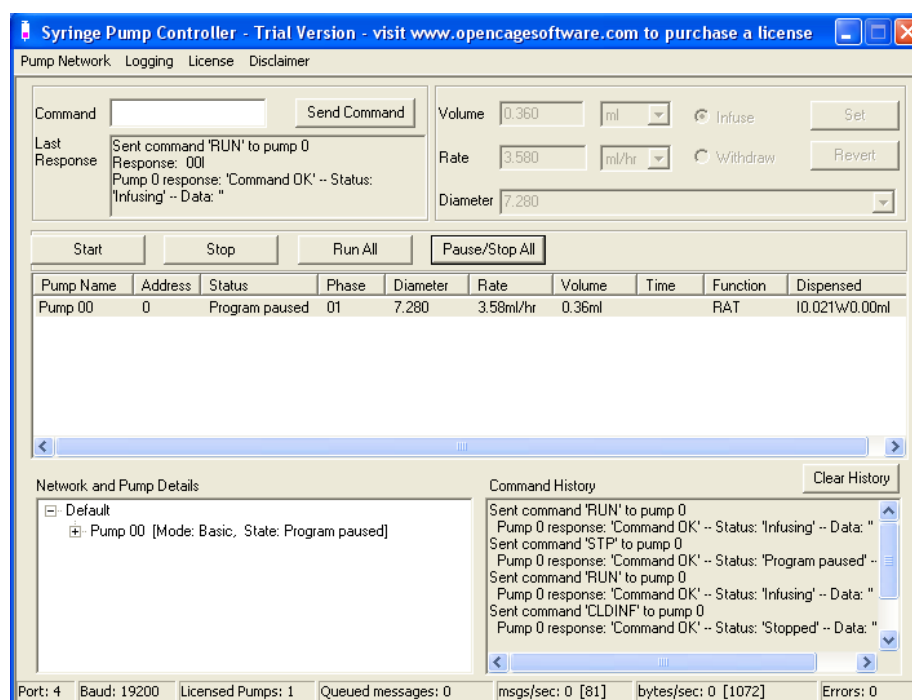
Figure 14: Syringe pump controller program interface

Different tocopherol release modes were simulated using different combinations of tocopherol concentrations and tocopherol diffusivities in the films, which were estimated in the Matlab^{TM} program, and then programmed in the syringe pump controlled program. Figure 15 is the example showing the release rate profiles of tocopherol under various conditions. It followed fast release rate in the beginning and slow release rate later. It is the situation of tocopherol release happened in real CRP films.
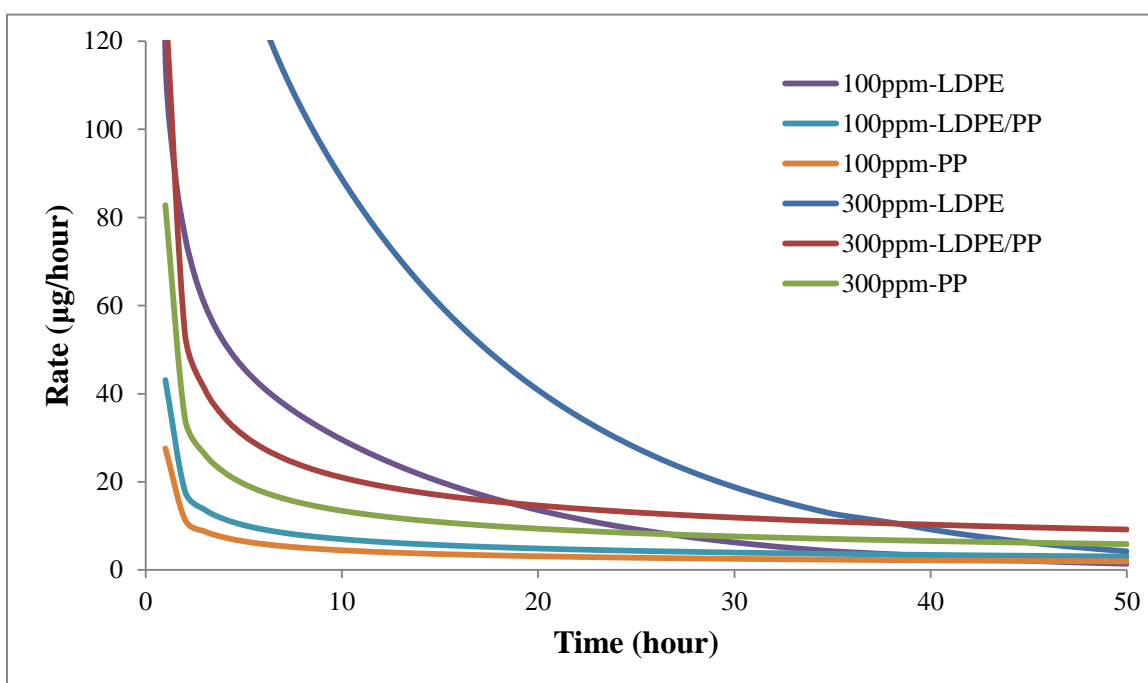
Figure 15: Release rate profile of different tocopherol concentrations with D$_{LDPE}$, D$_{LDPE/PP}$, D$_{PP}$

4.9.   Experimental Design for Lipid Oxidation Experiment

Lipid oxidation experiment was conducted to examine the antioxidant effect of tocopherol under different release modes. Instant addition experiment and syringe pump experiment were conducted respectively. Induction period of linoleic acid was used as an

indicator for its shelf life, and conjugated dienes was evaluated to determine the induction period.

4.9.1.   Tocopherol Stock Solutions Preparation

Different concentrations of tocopherol solutions were prepared by dissolving tocopherol into methanol using 100 ml Kimax volumetric flask. All the tocopherol stock solution were then flushed with nitrogen gas and stored at -18 ℃ before use. The amounts of tocopherol used for the stocked solution were list in Table 3.

Table 3: Different tocopherol stock solutions

Dissolved tocopherol amount (g)	Stock solution percentage (g/ml)
0.1	0.1%
0.3	0.3%
0.5	0.5%
1	1%
2	2%
3	3%
4	4%
5	5%

4.9.2. Lipid Oxidation Experiment

The experimental setup was developed base on the methods used by Wessling *et al.*[15] and Farhoosh *et al.* [51]. 250 ml Erlenmeyer flasks containing 10 g linoleic acid were exposed to air in the dark under constant rotary shaking at 100 rpm in an environmental chamber at 40 ℃. The linoleic acid in the flask was periodically sampled and the concentration of conjugated dienes in the samples determined optically at 234 nm (UV-1700 spectrophotometer, Shimazu Co., Kyoto, Japan). Samples were incubated until reaction had progressed well beyond the induction period and active oxidation was established.

4.9.3. Instant Addition Experiment

For instant addition experiment, 1 ml different tocopherol stock solutions were added into a 250 ml Pyrex flask containing linoleic acid all at once at time zero, respectively. No more tocopherol solution was added after that. Table 4 below showed different concentrations of tocopherol in linoleic acid. The flasks were exposed to air in dark, and rotary-shaken at 100 rpm at 40 ℃ in the environmental chamber. The linoleic acid samples were withdrawn periodically and measured by UV spectrophotometer at 234 nm to monitor the lipid oxidation progress.

Table 4: Different concentrations of tocopherol in linoleic acid

Concentration of tocopherol for 1 g linoleic acid (ppm)	Linoleic acid (g)	Tocopherol stock solution (g/ml)	Used volume of stock solution (ml)
0	10	-	-
100	10	0.1%	1
300	10	0.3%	1
500	10	0.5%	1
1000	10	1%	1
2000	10	2%	1
3000	10	3%	1
4000	10	4%	1
5000	10	5%	1

4.9.4. Syringe Pump Experiment

NE-4000 Double Syringe Pump (New Era Pump Systems, Inc., NY, USA) was used to simulate the release of tocopherol from different CRP films. Delivery rates were controlled by the syringe pump program using the simulated date. Two 1 ml syringes were filled up with tocopherol stock solution, placed on the syringe holder block, and driven by syringe pump program. A plastic tube was connected with the syringe and dip into linoleic acid. Tocopherol solution was then continuously infused into linoleic acid by syringe pump. The whole setup was in the environmental chamber at 40 ℃. Figure 16

showed the experimental setup for syringe pump experiment.
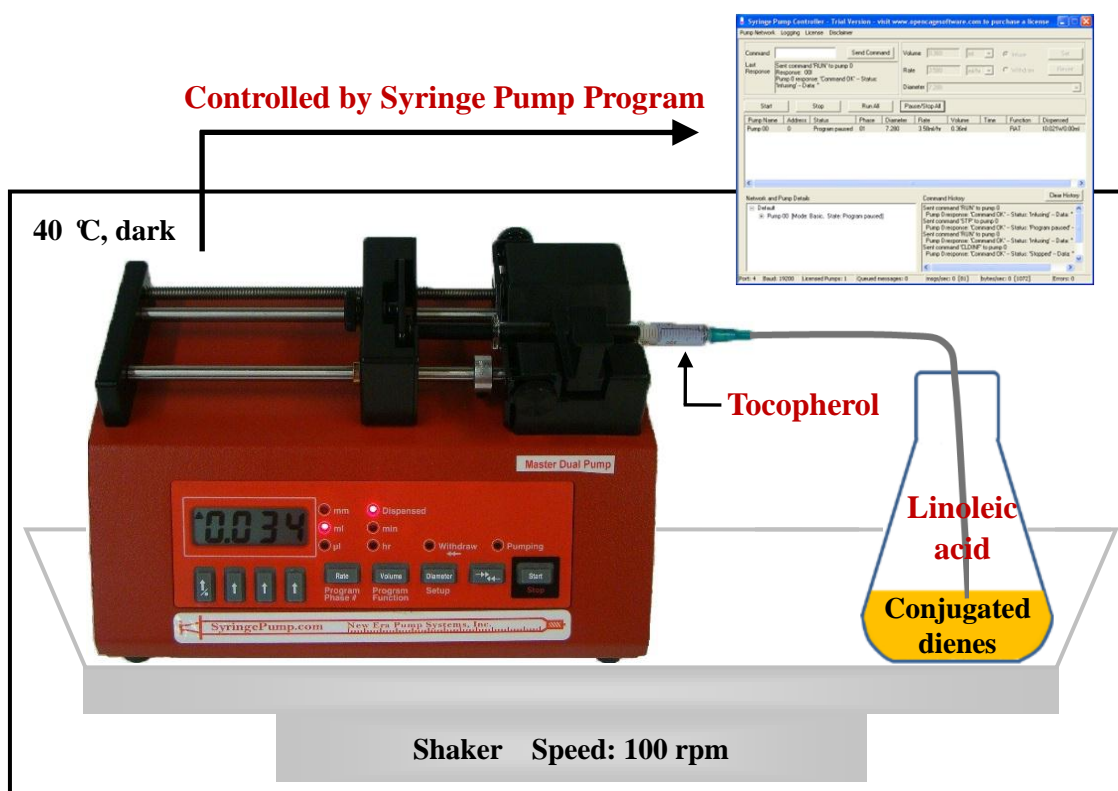
Figure 16: Syringe pump experiment setup

Table 5 listed different combinations of diffusivity of tocopherol concentration tested in syringe pump experiment. Tocopherol concentration was based on 1 g linoleic acid.

Table 5: Experimental runs for syringe pump experiment

Diffusivity	Tocopherol Concentration (ppm)	Linoleic acid (g)
D_{LDPE}, 40 ℃	100	10
	300	10
$D_{LDPE/PP}$, 40 ℃	100	10

4.10. Analytical Method for Lipid Oxidation Evaluation

Conjugated dienes was evaluated to determine the induction period of linoleic acid. The measurement method of conjugated dienes in linoleic acid was based on Shaker *et al.*[52] and Ng *et al.* [53]. 0.01 g linoleic acid sample was withdrawn periodically and dissolved in 5 ml cyclohexane, and then diluted for 25 times. Absorbance of the diluted solution was measured by UV spectrophotometer at 234 nm. Concentration of conjugated dienes (mol/l linoleic acid) was calculated based on its molar absorptivity of 26,000 l/(mol • cm).

4.11. Data Analysis

All experiments were done at least in duplicates. Average values were used in all tables and figures. Standard errors were used for error bars. Nonlinear regression was used for diffusion coefficient estimation by Matlab[TM] (version R2009a, Matlab[TM], Inc.).

5.   RESULTS & DISCUSSIONS

5.1.   Controlled Release Packaging Films Production

Three different CRP films containing tocopherol were produced using the Collin coextrusion cast line at Berry Plastics (Chippewa Falls, WI, USA) in July 2010. Films were stored in aluminum foil bags flushed with nitrogen before shipped to Rutgers University packaging laboratory for analysis. Table 6 listed detail conditions of film processing. The analysis for tocopherol containing films was discussed below.

Table 6: Processing parameters for tocopherol containing CRP films

Film	Temperature (°F)	Screw Speed (rpm)
LDPE	420	50
LDPE/PP	460	50
PP	480	50

5.2.   Physical Analysis of Films

Several physical properties of three CRP films were analyzed. The thickness of the films was measured in Rutgers; thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC) analysis and transmission Fourier transform infrared spectrometry (FTIR) analysis were conducted in Berry Plastics.

5.2.1.   Thickness

Thickness was measured by Digitrix II Micrometers (Fowler NSK, Japan). Twenty

points were selected randomly for each type of films. The average value was calculated

as the thickness of the films. The results were listed in Table 7.

Table 7: Thickness of three CRP films

	LDPE	LDPE/PP	PP
Thickness (mil)	4.285	3.6875	3.945
Thickness (m)	1.09×10^{-04}	9.37×10^{-05}	1.00×10^{-04}

5.2.2.　Cross Section Image

The cross section images of three films were taken by optical microscopy as shown

in Figure 17. LDPE tocopherol containing CRP film appeared as a monolayer film.

LDPE/PP blend tocopherol containing CRP film appeared as a monolayer also, but there

were some stripes appeared possibly indicating nonhomogeneous regions, which may due

to uneven mixing of LDPE and PP resins. PP tocopherol containing CRP film appeared as

a monolayer film.

| LDPE-Tocopherol Film | LDPE/PP-Tocopherol Film | PP-Tocopherol Film |

Figure 17: Cross section image of three films

5.2.3. TGA Analysis

TGA analysis found three degradation transitions for LDPE tocopherol containing CRP film, three degradation transitions for LDPE/PP blend tocopherol containing CRP film, and one degradation transition for PP tocopherol containing CRP film. Following figures showed TGS analysis for these three films.
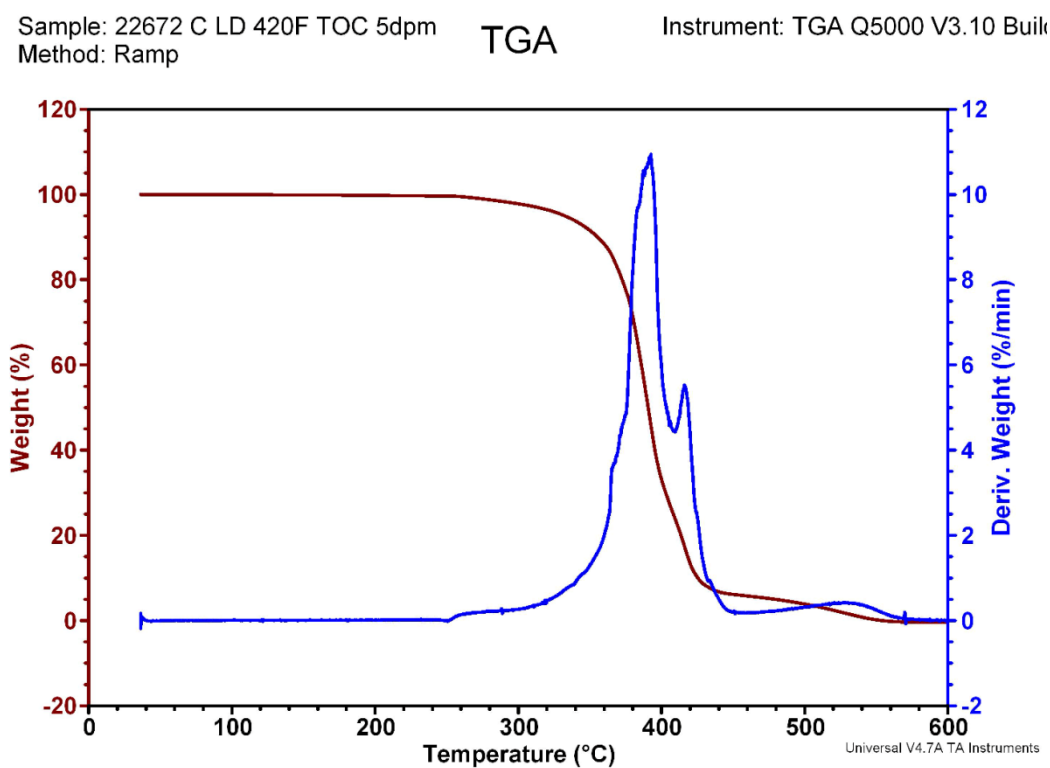
Figure 18: TGA analysis for LDPE tocopherol containing CRP film

Figure 19: TGA analysis for LDPE/PP blend tocopherol containing CRP film

Figure 20: TGA analysis for PP tocopherol containing CRP film

5.2.4. <u>DSC Analysis</u>

DSC analysis found LDPE in LDPE tocopherol containing CRP film; LDPE and hPP in LDPE/PP blend tocopherol containing CRP film; and hPP in PP tocopherol containing CRP film. Table 8 listed the DSC results of T_m and T_c for three films. Figure 21 - Figure 23 showed the DSC analysis.

Table 8: DSC results for three CRP films

Film	Material	T_m (℃)	T_c (℃)
LDPE	LDPE	106.2	96.7
LDPE/PP	LDPE	106.0	96.2
	PP (shoulder peak)	146.7	
	hPP	159.9	119.5
PP	PP (shoulder peak)	147.8	
	hPP	161.0	121.2

Sample: 22672 C LD 420F TOC
Method: Heat/Cool/Heat
DSC
Instrument: DSC Q2000 V24.8 Buil
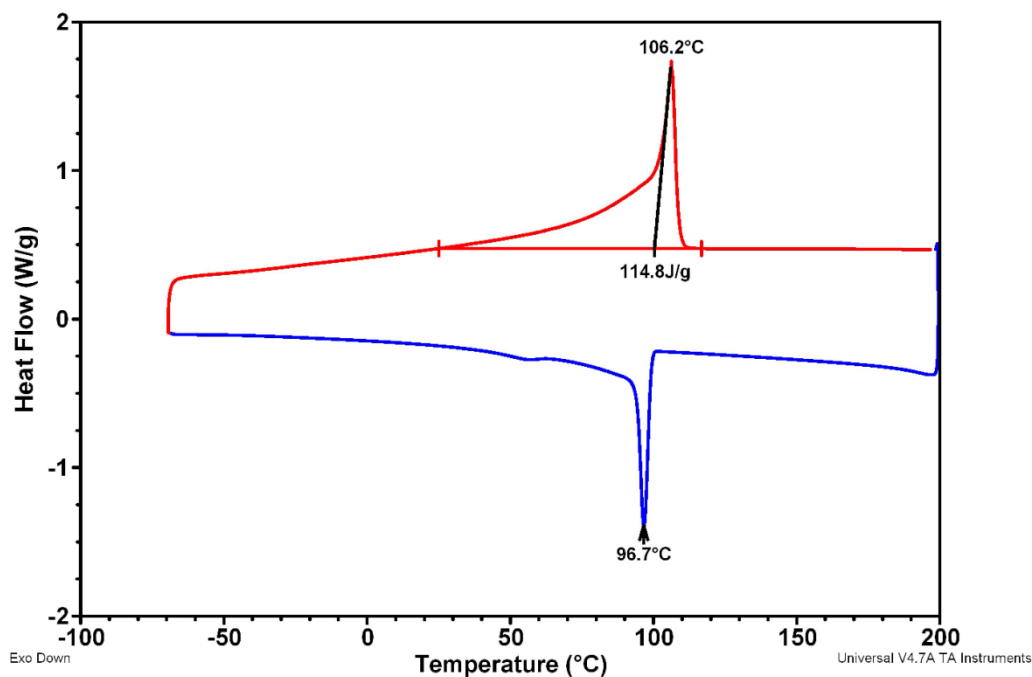
Figure 21: DSC analysis for LDPE tocopherol containing CRP film

Sample: 22672 A LD/PP 460F TOC
Method: Heat/Cool/Heat
DSC
Instrument: DSC Q2000 V24.8 Buil
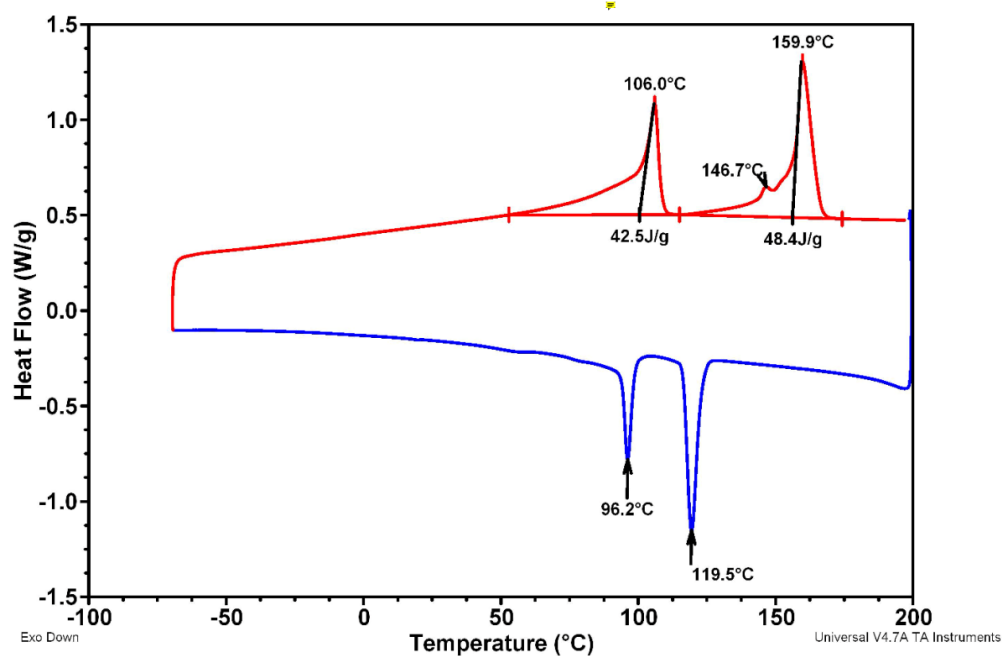
Figure 22: DSC analysis for LDPE/PP blend tocopherol containing CRP film
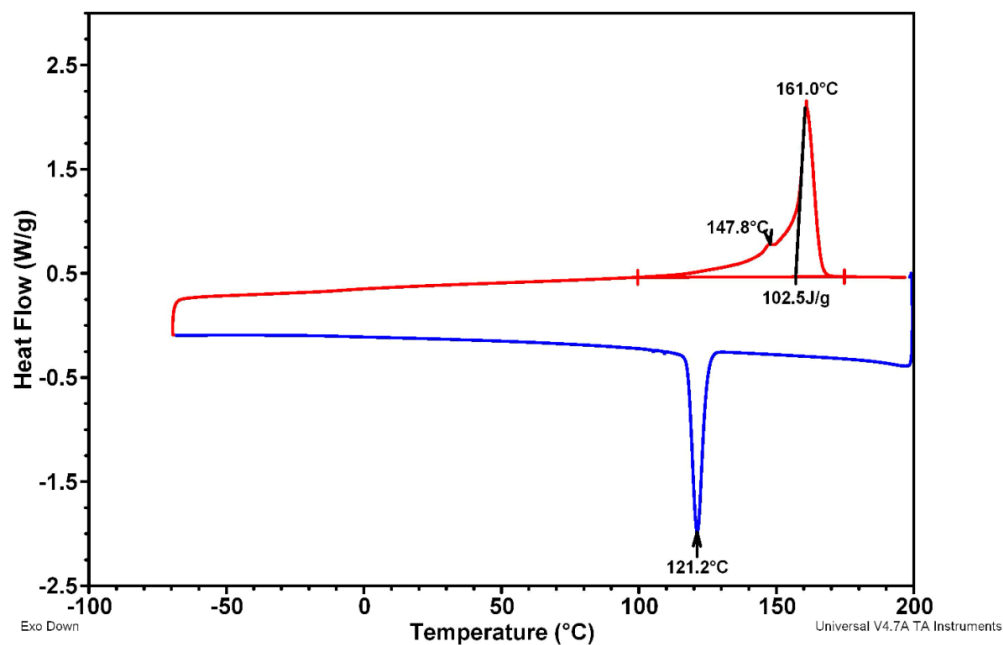
Figure 23: DSC analysis for PP tocopherol containing CRP film

5.2.5. Transmission FTIR Analysis

LDPE Transmission FTIR analysis (Figure 24 - Figure 26) found LDPE, LDPE and PP, and hPP in LDPE, LDPE/PP blend and PP films, respectively. However, it didn't detect any tocopherol in the films, which may due to the instrument detection limits.
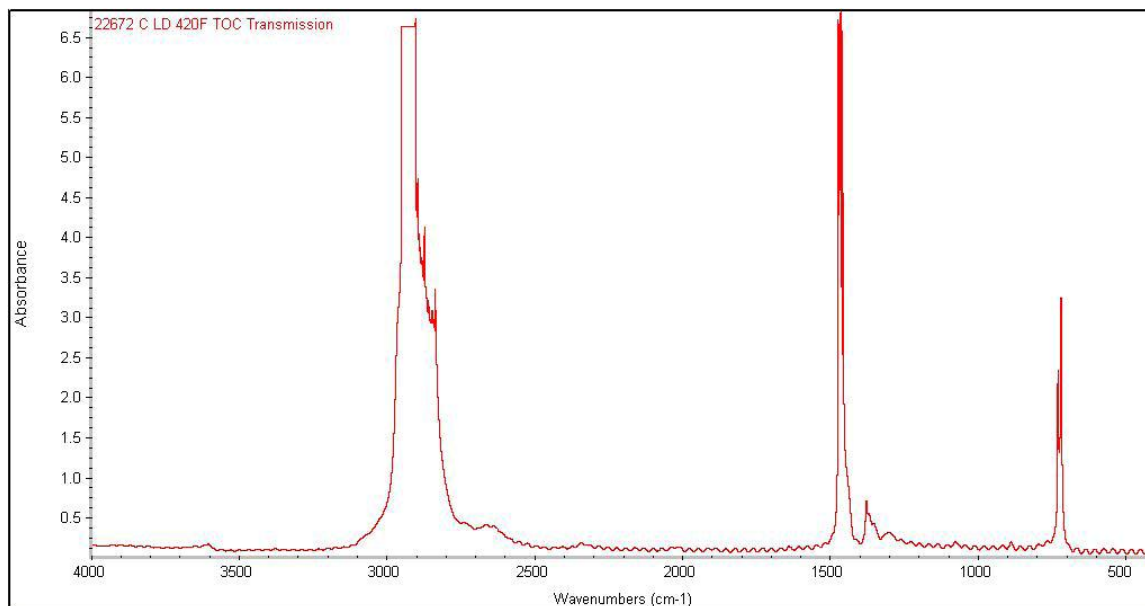
Figure 24: Transmission FTIR analysis for LDPE tocopherol containing CRP film
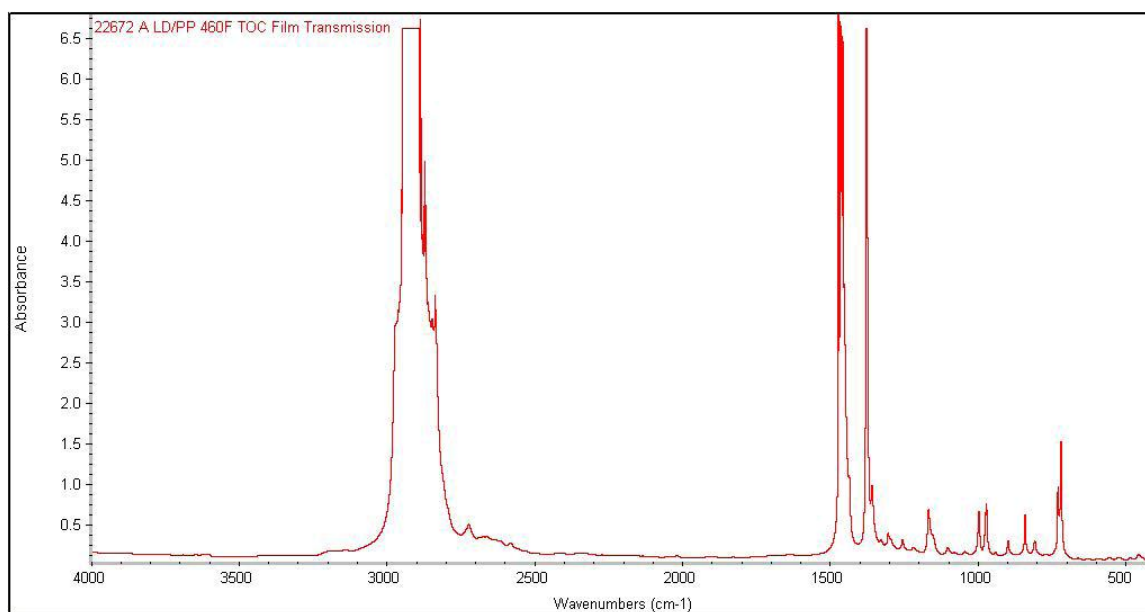
Figure 25: Transmission FTIR analysis for LDPE/PP blend tocopherol containing CRP film
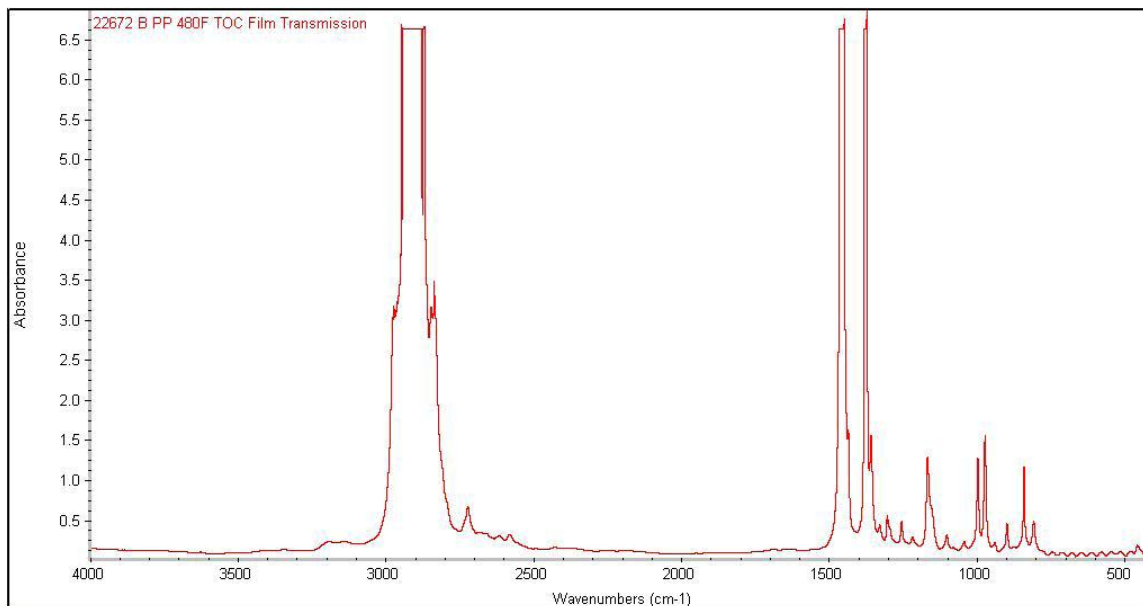
Figure 26: Transmission FTIR analysis for PP tocopherol containing CRP film

5.3. Total Extraction of Tocopherol from Films

Shake-flask extraction method was used in total extraction study. Methylene chloride was used to extracted total available tocopherol from films because of its ability to dissolve a wide range of organic compounds [54]. The results (Figure 27) showed that more than 90% tocopherol was extracted from the films (Table 9). The loss may be due to processing loss or chemical bonding of tocopherol with polymers. As to others' work on total extraction, Wessling *et al.* found the total extraction of α-tocopherol in LDPE was less than 40% [15], and Sir ó *et al.* showed that the total extraction of α-tocopherol in their LDPE films were about 75% and 80% [14]. Different results may be due to different film processing methods and conditions. In this research, PP has the highest amount of extractable tocopherol in the film among three films. This may be explained that the

crosslink of PP is higher than LDPE, so it can trap more tocopherol molecules in the film matrix, and make them stable under the severe processing conditions. The result suggested that the extrusion process can be used to impregnate tocopherol into a plastic film without substantial loss.
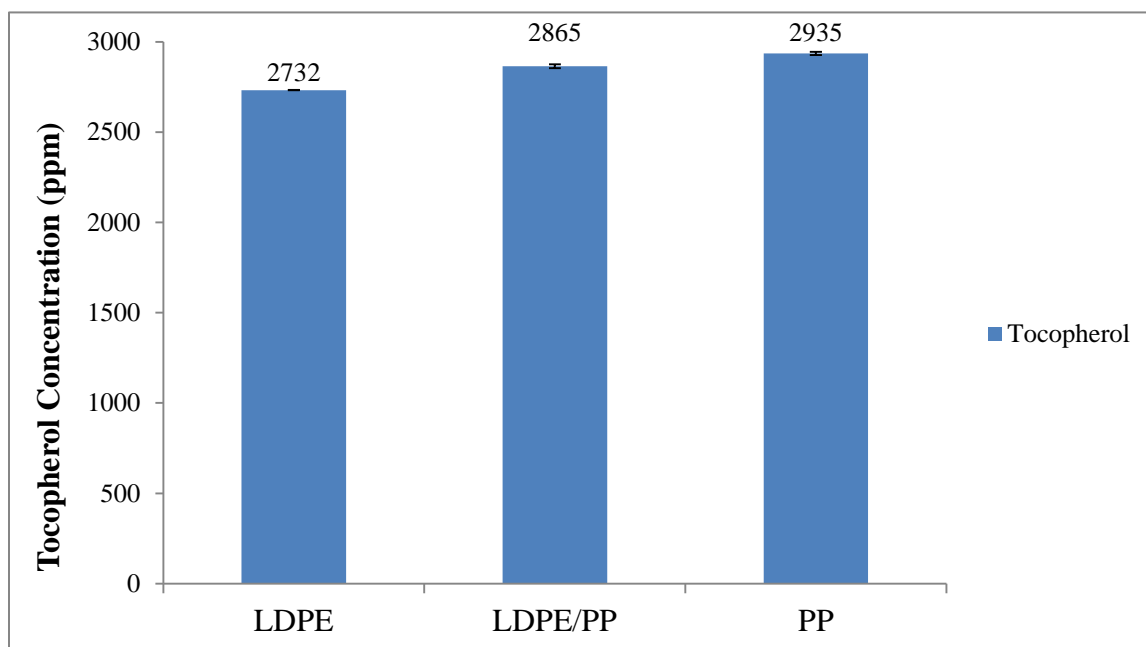
Figure 27: Total extraction of tocopherol from CRP films

Table 9: Total extraction of tocopherol from films

Film	Blending ratio (LDPE/PP, w/w)	Initial concentration of tocopherol added into films (ppm)	Actual concentration of available tocopherol in films (ppm)	Extraction percentage (%)
LDPE	100/0	3000	2732 ± 1	91%
LDPE/PP	50/50	3000	2865 ± 11	96%
PP	0/100	3000	2935 ± 9	98%

5.4.   Release Kinetics of Tocopherol from Films

During the migration of tocopherol from package to food for direct contact, there are three steps: diffusing in the polymers, partitioning at the package/food interface and dissolving in the food. The release study experiment was used to study the tocopherol release kinetics during the migration. 95% ethanol was selected as a food simulant to simulate fatty food based on two reasons: (1) 95% ethanol was a good food simulant for fatty foods [50]; (2) There's no tocopherol degradation during release study using 95% ethanol. The release studies of tocopherol from different films were conducted at 30, 40 and 50 ℃ with constant rotary shaking (100 rpm) in dark. The diffusion of tocopherol in LDPE was much faster than LDPE/PP blend and PP films. Figure 28 showed all the release study experiment results under different conditions.
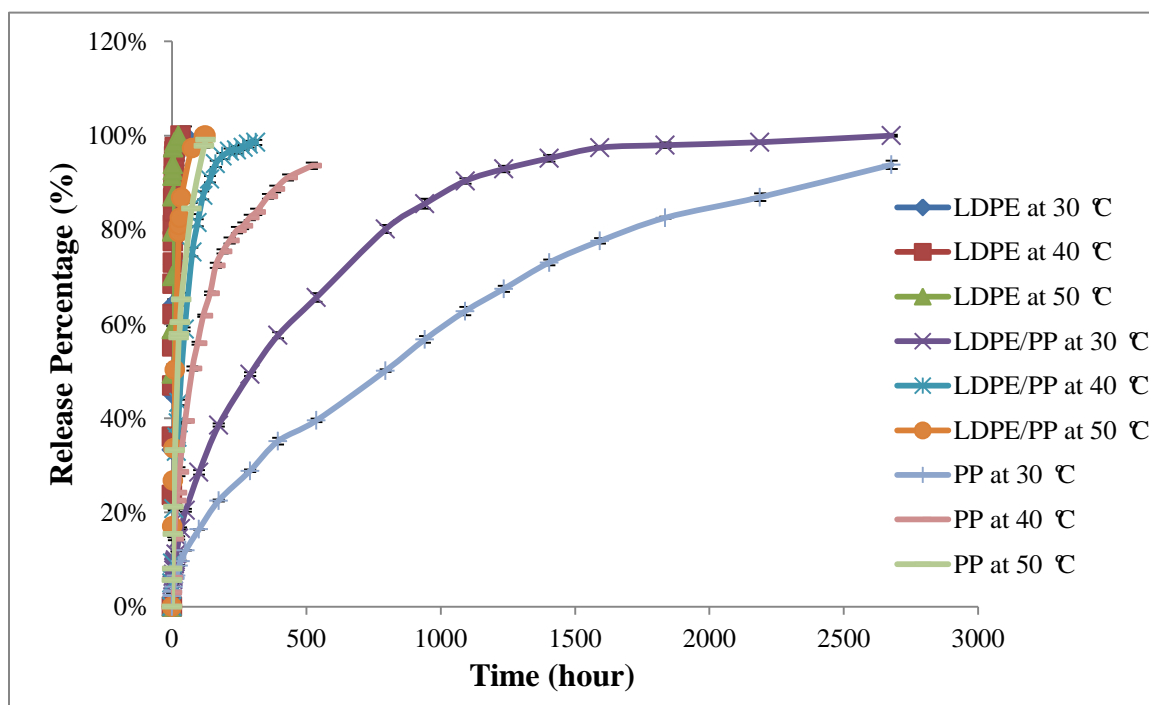
Figure 28: Release study of tocopherol from CRP films using 95% ethanol

Figure 29 - Figure 31 showed release of tocopherol from three films at different temperatures. The release trend of tocopherol followed fastest in LDPE, then LDPE/PP blend, and slowest in PP at all 30, 40 and 50 ℃. After 9 hours, 90% tocopherol released from LDPE at 30 ℃, however, the same amount of tocopherol released from LDPE/PP blend and PP films until 1090 hours (~45 days) and 2500 hours (~104 days), respectively. This can be explained that PP has more crystal regions than LDPE, thus the tortuosity of tocopherol pathway in PP is much longer than in LDPE, which contributed to longer release period. By manipulating the composition of polymers could change tocopherol diffusion to achieve desired release for various food products. The effect of polymer composition on the tocopherol release has also been reported by Obinata [54] and Zhu [17]. The wide range of tocopherol diffusion provided us broad choices of tocopherol release to fit different food products.
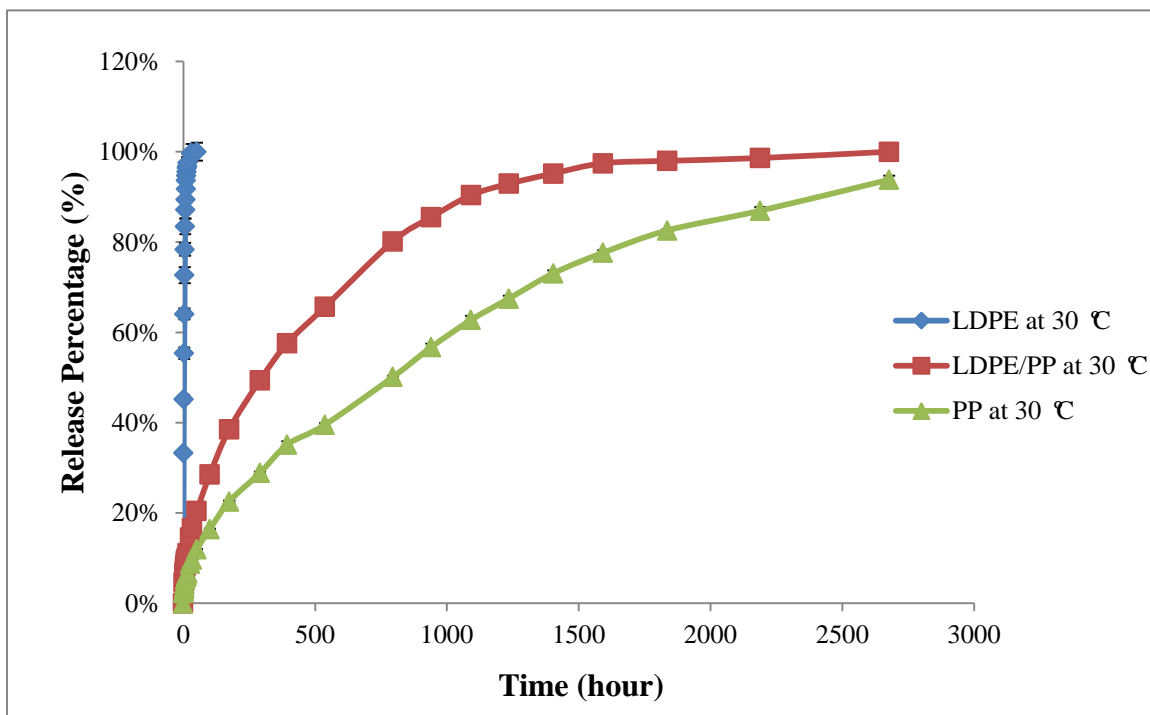
Figure 29: Release study of tocopherol from CRP films using 95% ethanol at 30 ℃
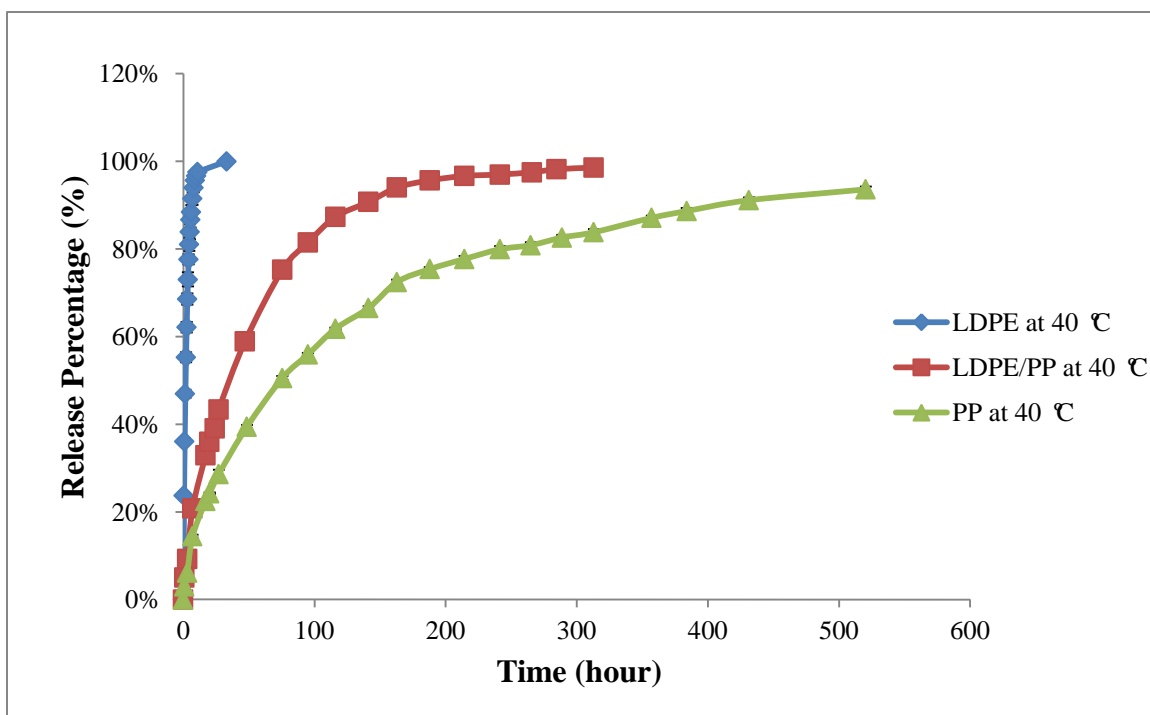
Figure 30: Release study of tocopherol from CRP films using 95% ethanol at 40 ℃
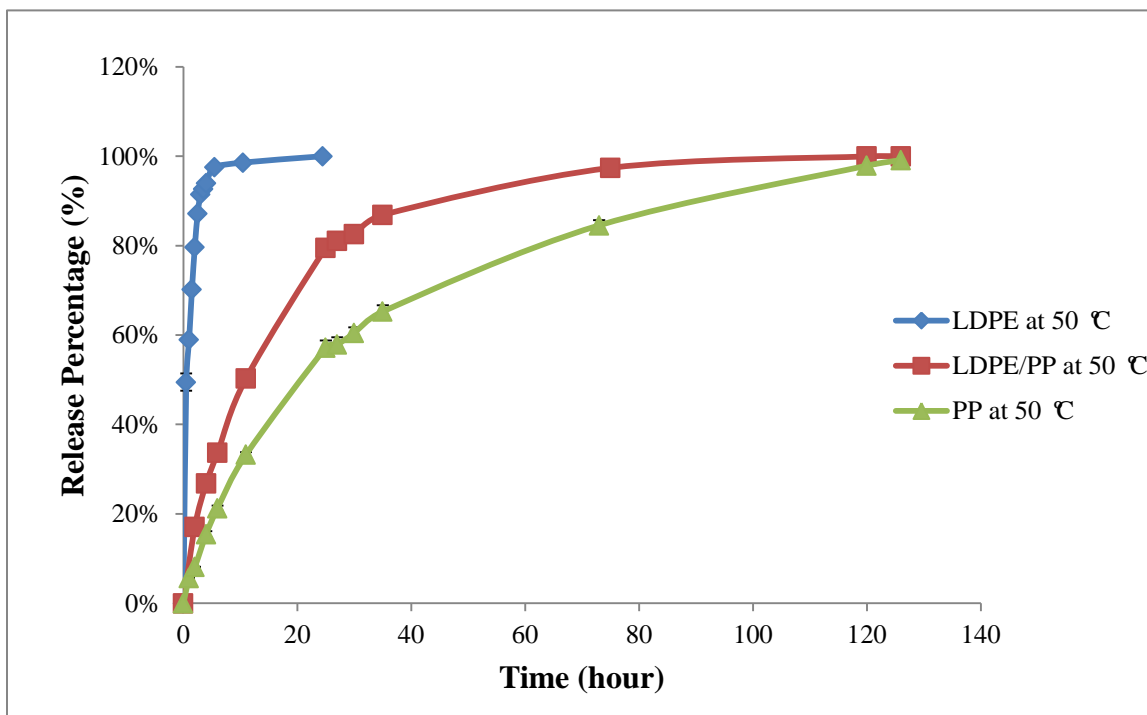
Figure 31: Release study of tocopherol from CRP films using 95% ethanol at 50 ℃

The diffusion of tocopherol also increased when temperature increased as shown in Figure 32 - Figure 34. Especially for PP/tocopherol film (Figure 34), the diffusion increased much greater than LDPE/tocopherol film for same temperature gradient. The temperature dependence of diffusivity was studied in the Section 5.6.
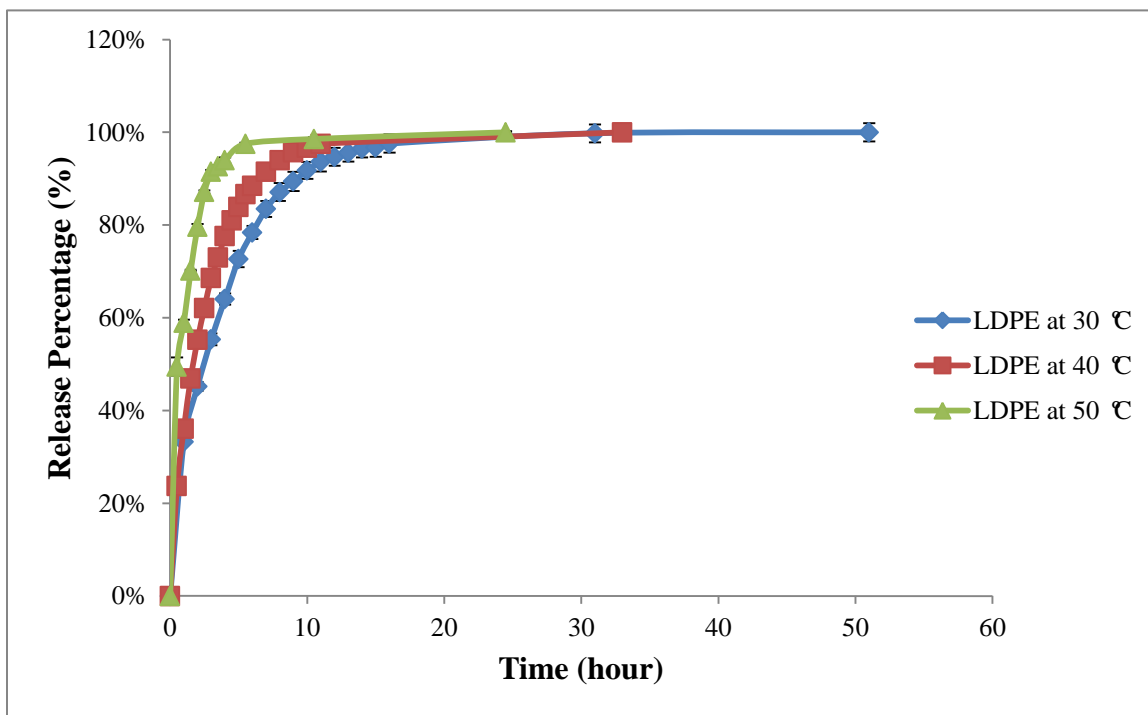
Figure 32: Release study of tocopherol from LDPE CRP film using 95% ethanol
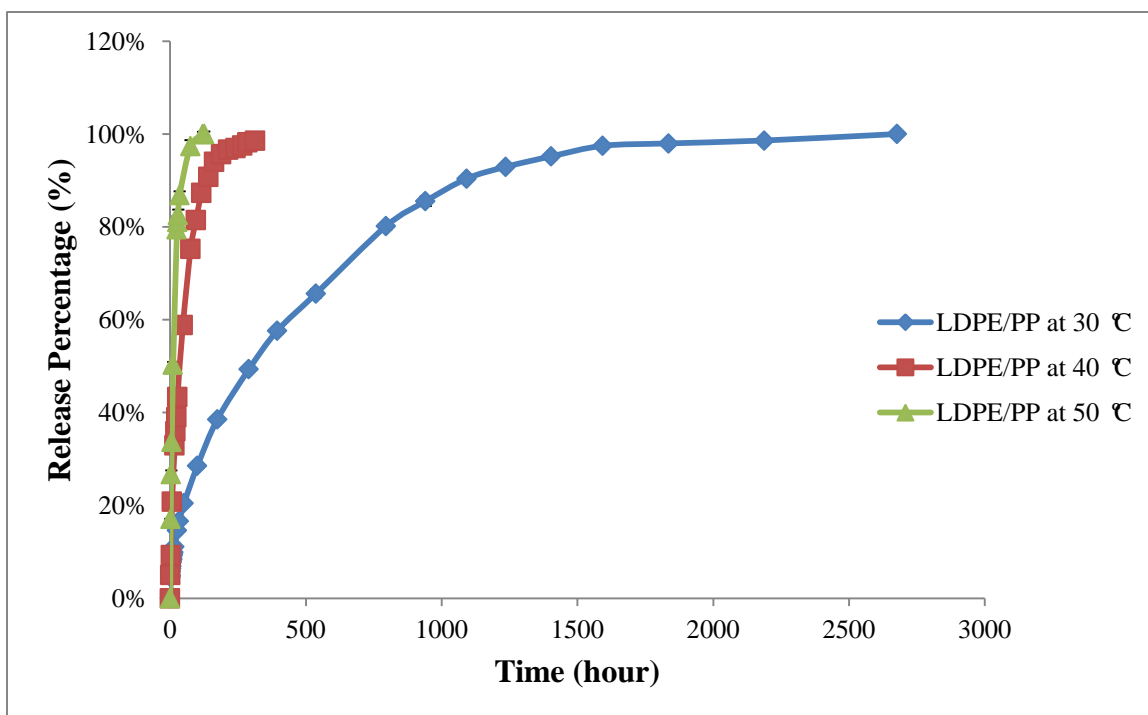
Figure 33: Release study of tocopherol from LDPE/PP CRP film using 95% ethanol
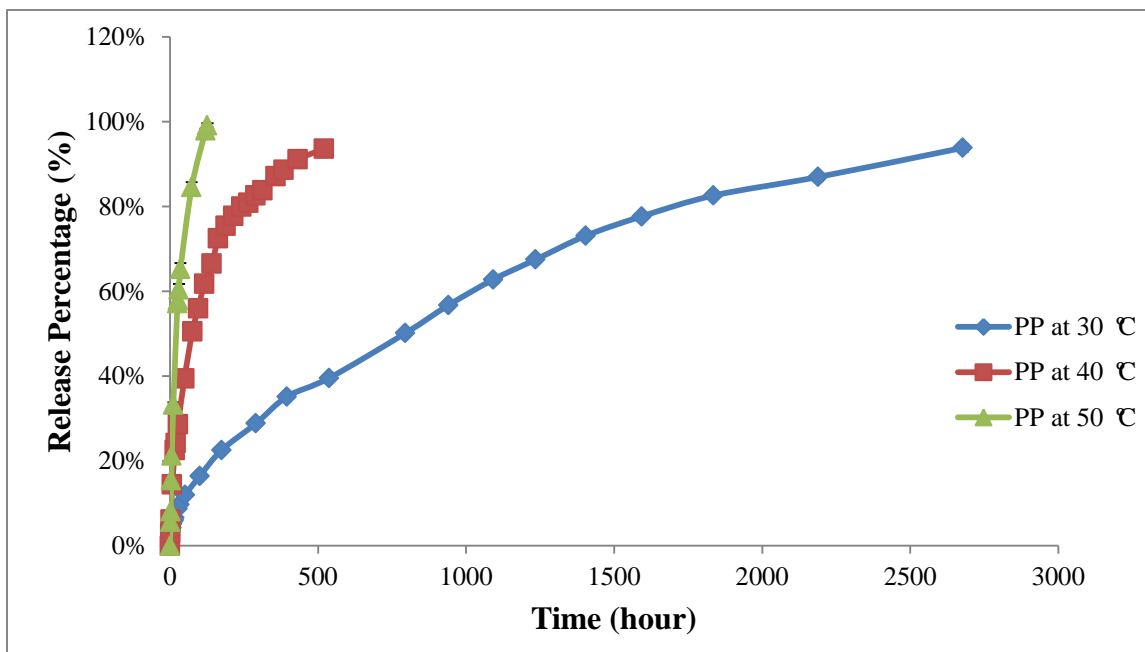
Figure 34: Release study of tocopherol from PP CRP film using 95% ethanol

5.5. Estimation of Tocopherol Diffusivity

Matlab^{TM} program was written for diffusivity estimation based on equation 1 in Matlab^{TM} R2009a. The program used curve fitting method to fit the model to the data. The algorithm is based on golden section search and parabolic interpolation [55]. Nonlinear regression was used to calculate the best-fit diffusivity value, and diffusivity was determined by the minimum sum of the squared of errors (SSE) between the experimental and estimated values. Actual experimental data and estimated diffusivity data were compared in Figure 35, which demonstrated that the modeled diffusivity of tocopherol in LDPE fitted actual experiment data very well. Individual comparisons on actual experiment data and estimated diffusivity data were shown blow that red dots represented experimental data and blue line represented estimated data.
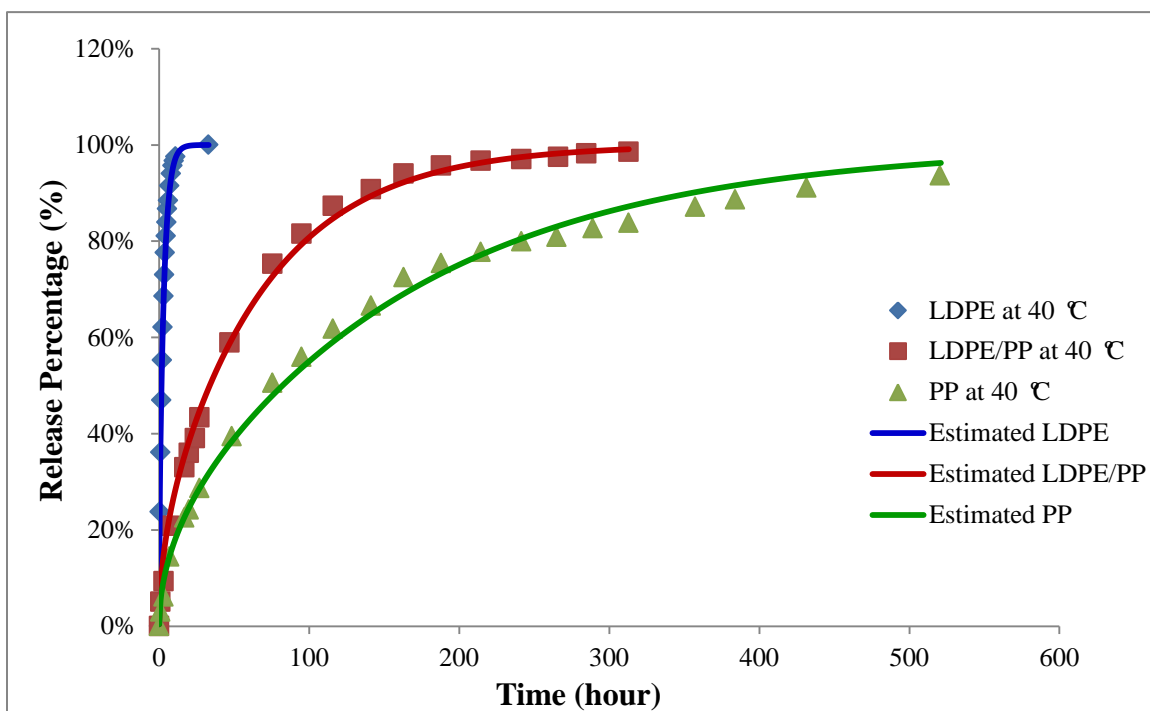
Figure 35: Diffusivity estimation of tocopherol in CRP films at 40 ℃
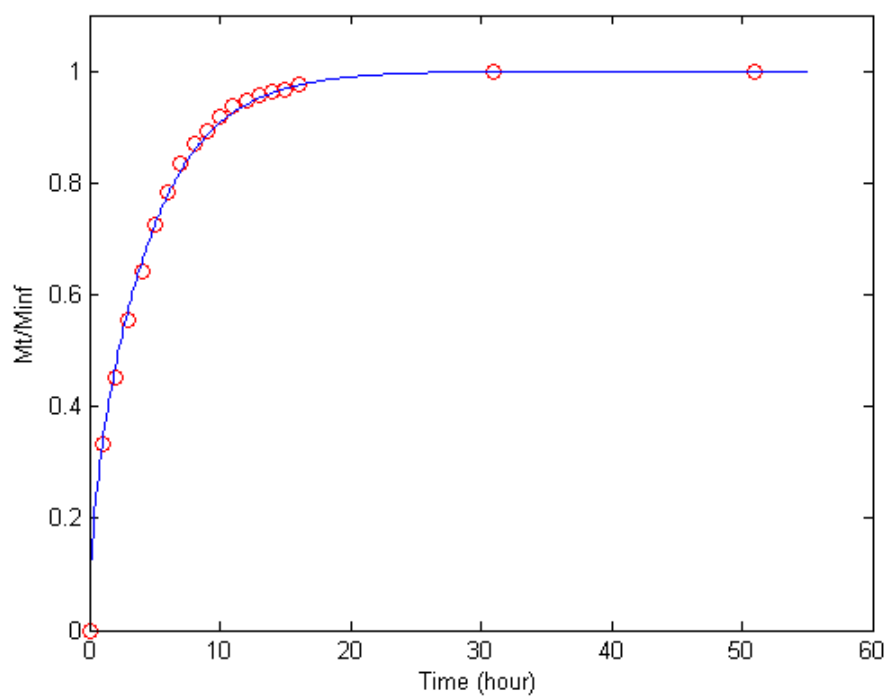
Figure 36: Diffusivity estimation of tocopherol in LDPE films at 30 ℃
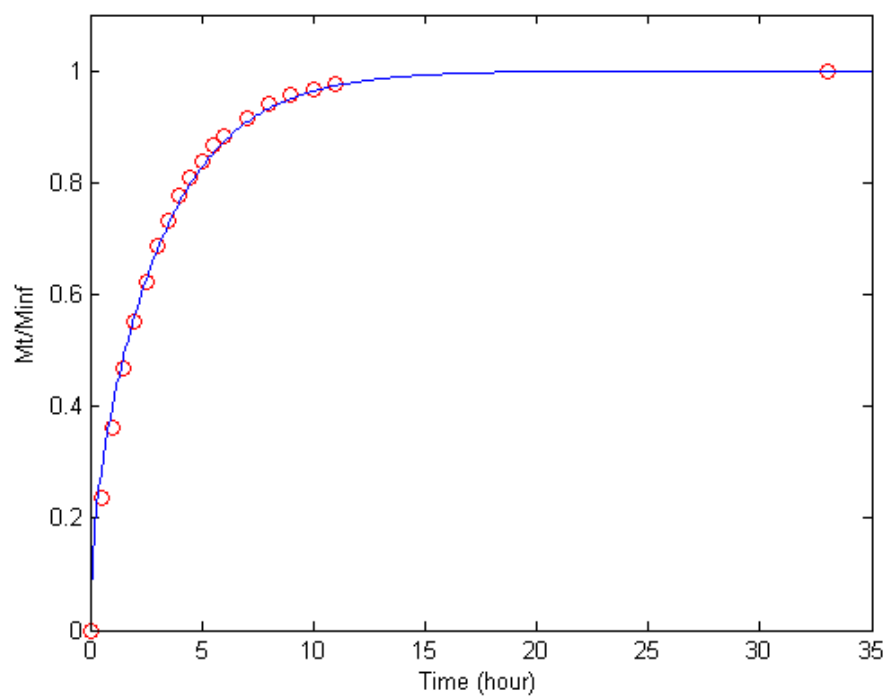
Figure 37: Diffusivity estimation of tocopherol in LDPE films at 40 °C
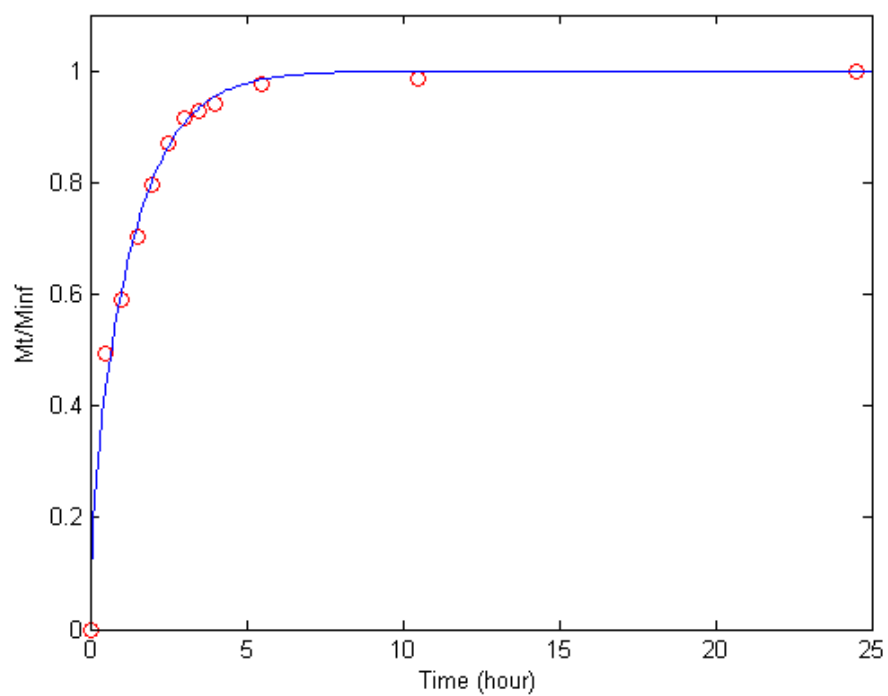
Figure 38: Diffusivity estimation of tocopherol in LDPE films at 50 °C
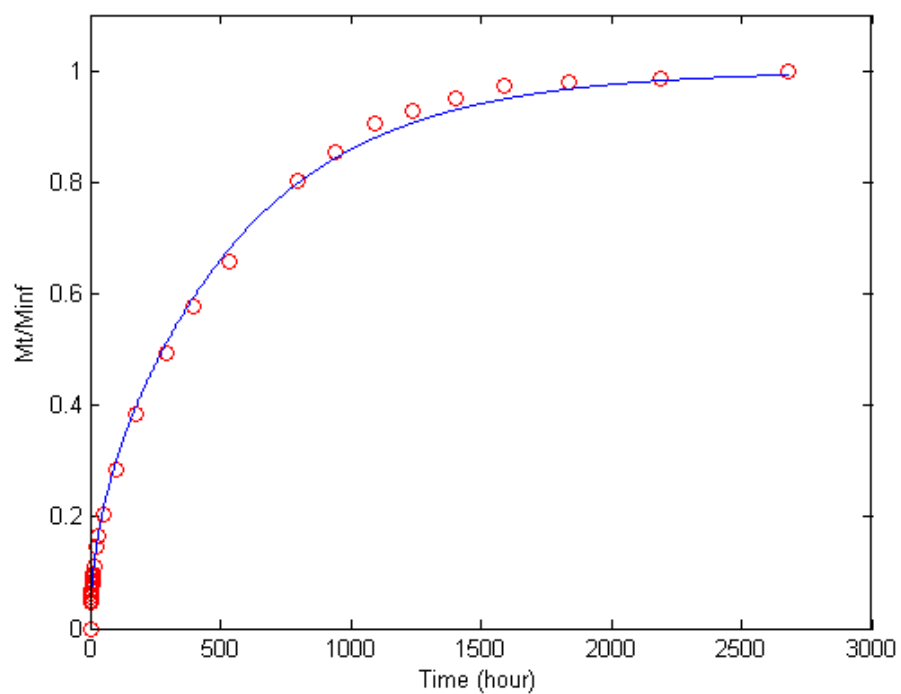
Figure 39: Diffusivity estimation of tocopherol in LDPE/PP films at 30 ℃
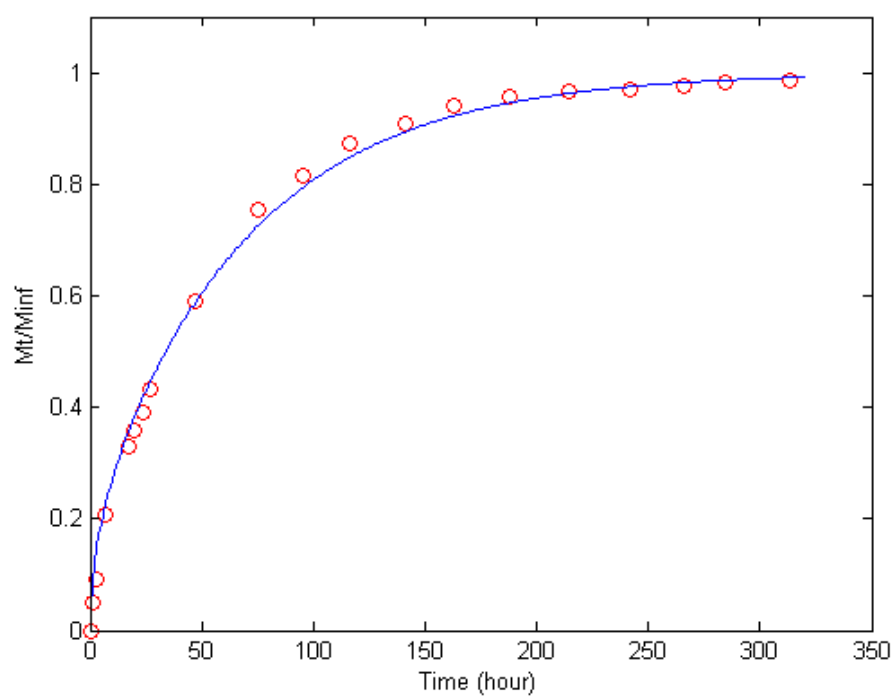
Figure 40: Diffusivity estimation of tocopherol in LDPE/PP films at 40 ℃
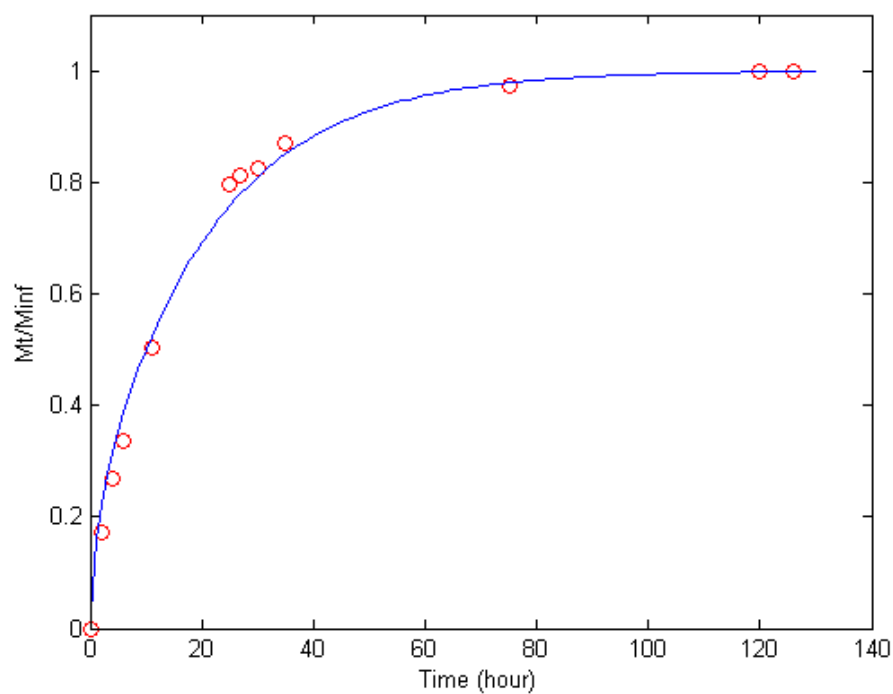
Figure 41: Diffusivity estimation of tocopherol in LDPE/PP films at 50 ℃
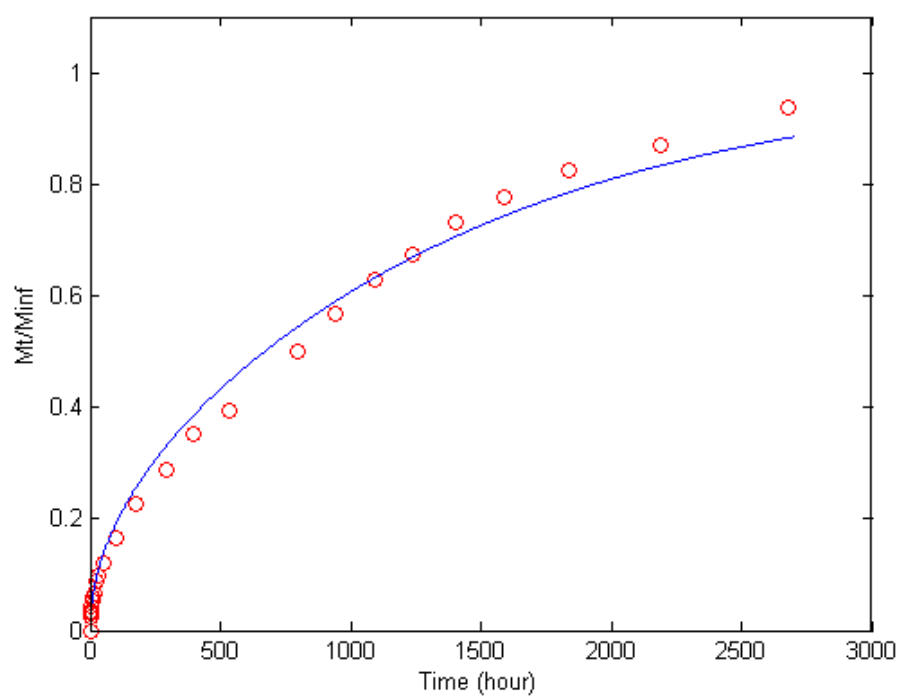
Figure 42: Diffusivity estimation of tocopherol in PP films at 30 ℃
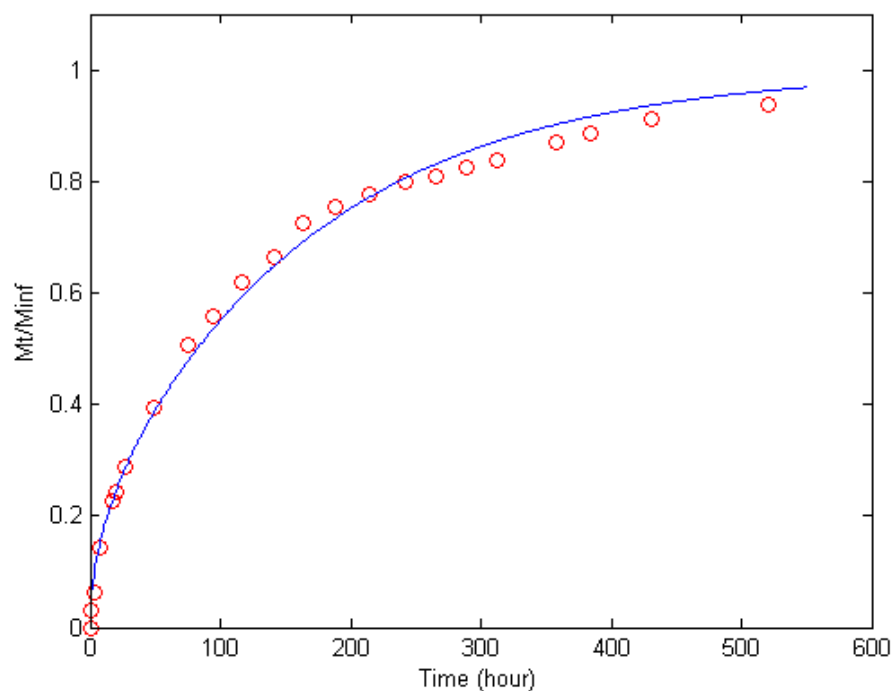
Figure 43: Diffusivity estimation of tocopherol in PP films at 40 ℃
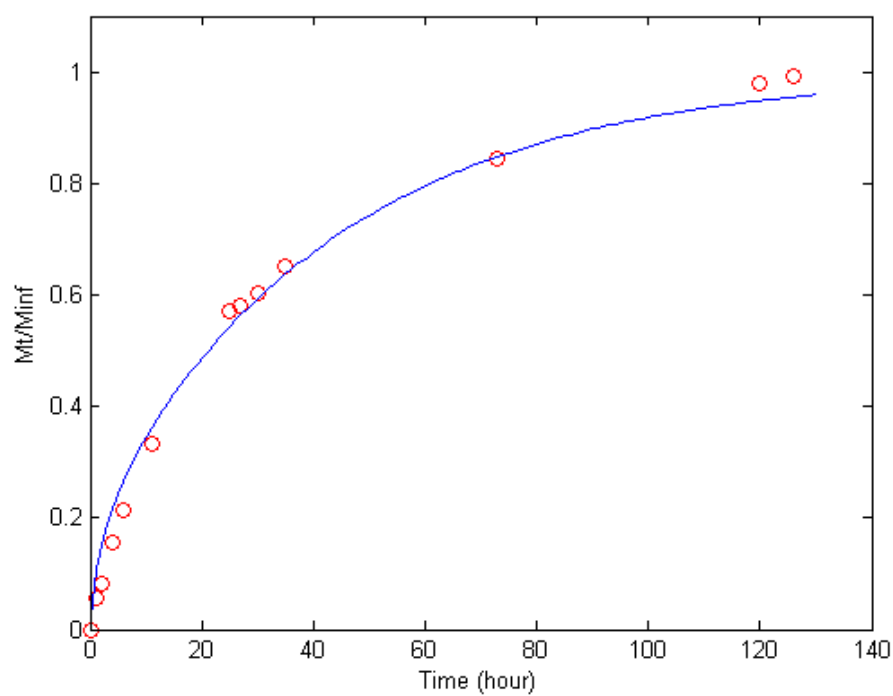
Figure 44: Diffusivity estimation of tocopherol in PP films at 50 ℃

Estimated diffusivity values for three films at different temperatures were listed in Table 10. The diffusion of tocopherol in LDPE/PP blend was not the average value of the ones in LDPE and PP. It seems that diffusion of tocopherol was dominated by PP. The reason behind it may be due to the chemical affinity of tocopherol with the polymers or the polymer crystallinity differences [1, 56].

Table 10: Estimation of tocopherol diffusivity in CRP films at various temperatures

Temperature	Diffusivity Estimation (m^2/s)		
	LDPE	LDPE/PP	PP
30 ℃	7.20×10^{-14}	4.31×10^{-16}	2.04×10^{-16}
40 ℃	1.03×10^{-13}	3.56×10^{-15}	1.67×10^{-15}
50 ℃	2.39×10^{-13}	1.19×10^{-14}	6.50×10^{-15}

Table 11 showed a summary of tocopherol diffusivity values in LDPE at 30 ℃ from different research. In this research, the diffusivity was 7.20×10^{-14} m^2/s by coextrusion, which was around the same order of magnitude with other research by blow extrusion (from 3.06×10^{-15} to 5.11×10^{-14} m^2/s).

Table 11: Diffusivity of tocopherol comparison

Polymer	Processing Method	Temperature (°C)	D(m²/s)
LLDPE* + Ziegler-Natta [57]	blown-extrusion	30	4.2×10^{-14}
LDPE (HDPE + TiO2/EVOH/LDPE + 4% α-tocopherol) [58]	blown-extrusion	30	3.06×10^{-15}
LDPE + 20000 ppm tocopherol [59]	blown-extrusion	30	3.03×10^{-14}
LDPE + 40000 ppm tocopherol [59]	blown-extrusion	30	5.11×10^{-14}

*LLDPE: Linear low-density polyethylene

5.6. Temperature Dependence of Diffusivity

Temperature dependence of diffusivity was studied. Arrhenius activation energy model is used to describe the temperature effect on diffusivity:

$$D = D_0 exp\left(-\frac{E_a}{RT}\right) \quad \text{(Equation 3)}$$

where D is the diffusion coefficient (m²/s), D_0 is a constant (m²/s), E_a is activation energy for the diffusion (J/mol), R is universal gas constant (J/mol · K), and T is absolute temperature (K).

The Arrhenius plots were derived from logarithmic transform of Equation 3 (Figure 45). E_a were defined as the required energy for a migrant to move among the chains forming the polymer matrix [60], which were 8.5, 135.7 and 141.2 kJ/mol for LDPE,

LDPE/PP blend and PP films, respectively. The results for E_a indicated that less energy was required for tocopherol to move in LDPE than that in LDPE/PP blend and PP matrix. Graciano-Verdugo *et al.* reported that the E_a were 126.5 and 105.9 kJ/mol for 19.07 and 30.18 mg/g α-tocopherol containing LDPE films [59].
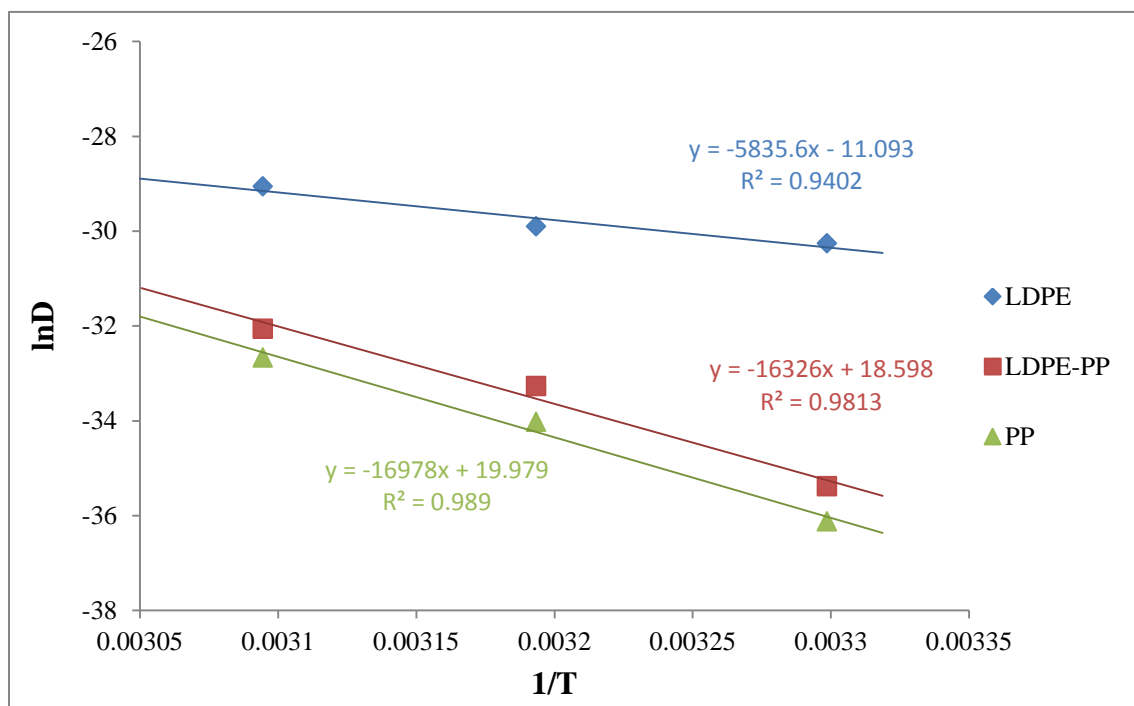
Figure 45: Diffusivity estimation of tocopherol for different temperatures using Arrhenius equation

Table 12 indicates significant effect of temperature on diffusion with the high correlation coefficient values ($r^2 > 0.94$) for each film. The greater the E_a is, the more sensitive the diffusivity to temperature changes.

Table 12: Activation energy and correlation value between diffusivity (lnD) and temperature (1/T) derived from Arrhenius plot

Film	Food Simulant	E_a (kJ/mol $\cdot$ K)	$D_0(m^2/s)$	r^2
LDPE	95% ethanol	48.5	5.07×10^{-3}	0.9402
LDPE/PP	95% ethanol	135.7	1.19×10^8	0.9813
PP	95% ethanol	141.2	4.75×10^8	0.9890

In summary, tocopherol-containing CRP films with LDPE, LDPE/PP blend and PP polymers were produced, and the retention of tocopherol in these films were more than 90% after processing. It is evident from the results that tocopherol was successfully impregnated into films by coextrusion. The diffusion of tocopherol in LDPE, LDPE/PP blend and PP followed the Fick's Law, and the diffusivities were estimated assuming Ficken diffusion in two dimensions diffusion for large migration times, which were from 2.04×10^{-16} m^2/s to 2.39×10^{-13} m^2/s at different temperatures. The tocopherol diffusion was fastest in LDPE, then in LDPE/PP blend and slowest in PP. Manipulating polymer composition can provides different tocopherol diffusion, thus different release of tocopherol for targeting food products, and it also confirmed LaCoste's work [2]. However, diffusivity value of tocopherol in LDPE/PP were of the same order of magnitude with the one in PP, but two order of magnitudes smaller than the one in LDPE at 30 and 40 $°C$. This may be explained that the crystallinity in PP dominated the diffusion of tocopherol.

5.7. Syringe Pump Simulation

Syringe pump is used to simulate the release of tocopherol from different CRP films. In the Section 5.5, the diffusivity values for tocopherol in different films were estimated, and the release happened in two dimensions, which was from release study results. In syringe pump simulation, syringe pump is mimicking the release of tocopherol from film into linoleic acid as a package. In other word, the release of tocopherol happened only in one direction, package to food. Therefore, in order to calculate the release percentage, Equation 2 was modified into:

$$\frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} exp\left[\frac{-D(2n+1)^2\pi^2 t}{4L_P^2}\right] \qquad \text{(Equation 4)}$$

where $M_{F,t}$ is the mass of the migrant in the food at a particular time t (s), $M_{F,\infty}$ is the mass of the migrant in the food at infinite time (in equilibrium), L_P (m) is the thickness of the film, D (m^2/s) is the diffusion coefficient of the migrant in the polymer, and t (s) is the time.

The thickness was followed the produced CRP films. Different diffusivity values and thickness were substituted into equation to calculate the release percentage, $\frac{M_{F,t}}{M_{F,\infty}}$, at time points.

Figure 46 is the simulation result of tocopherol release rate using different concentration and different films in syringe pump system. This profile simulated the release rate of tocopherol from real CRP films containing 3000 ppm tocopherol. Different

concentrations are used in the simulation. There is a concern that tocopherol may act as a

plasticizer in the film, so different concentration of tocopherol may change the diffusivity.

However, there's no enough evidence to support this point. Therefore, in this research, it

is assumed that concentration change did not bring other effects on tocopherol diffusivity

in the film. Due to the limitation of the syringe pump, the release behavior of tocopherol

from PP was to slow to program in the syringe pump.
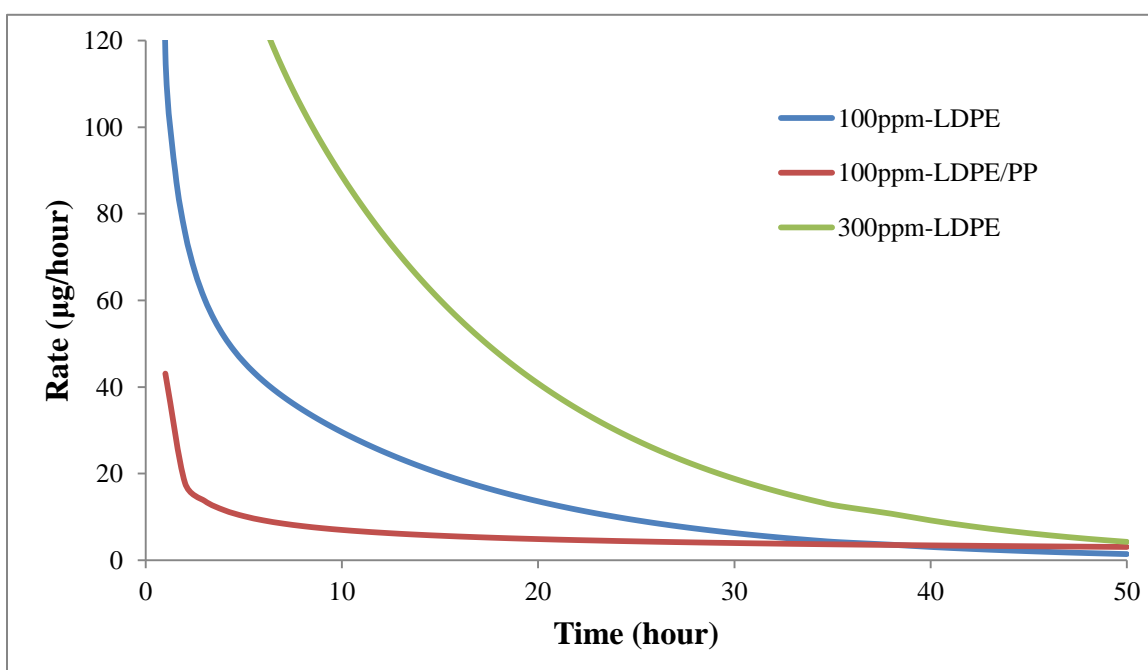
Figure 46: Release rate profile of tocopherol delivery by syringe pump system

Figure 47 is the plot of released tocopherol amount using different concentrations of

tocopherol in D_{LDPE} and $D_{LDPE/PP}$. It can tell us the amount of tocopherol released into

linoleic acid during the induction period. For example, we use certain tocopherol

concentration (100 ppm) delivered by different modes (D_{LDPE} and $D_{LDPE/PP}$), the total

released amount of tocopherol could be only half of the total amount delivering in

$D_{LDPE/PP}$ if the induction period is around 150 hours, while the total release amount of tocopherol delivering in D_{LDPE} could be the initial total amount of tocopherol.
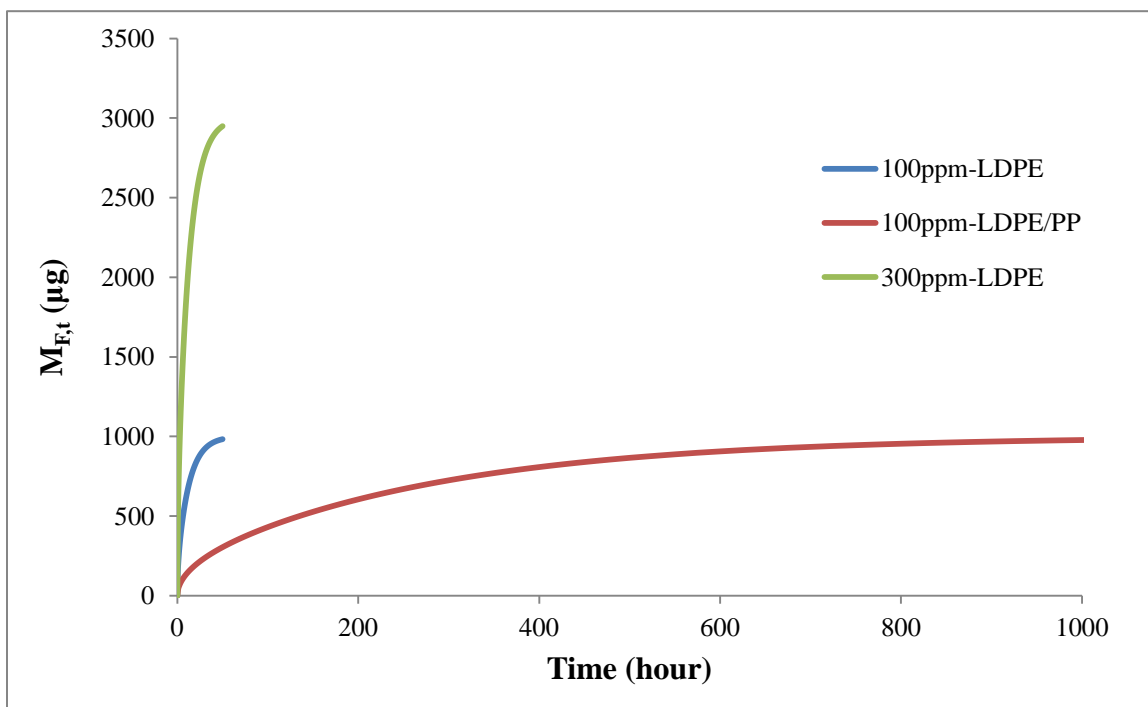
Figure 47: Release profile of tocopherol delivered by syringe pump using different concentrations in D_{LDPE} and $D_{LDPE/PP}$

In Summary, release profile of tocopherol from CRP was generated successfully using estimated diffusivity, various concentrations and syringe pump system. In order to mimic the real release situation of tocopherol from package to food, one dimension release was used in simulation instead of two dimensions release in release study. Release rate was faster in the beginning and slower later, and it dropped tremendously in the initial stage.

5.8.   Lipid Oxidation Experiment

5.8.1.   Lipid Oxidation Evaluation

Linoleic acid, a major component of vegetable oil, is used as a food model system to study the antioxidant effectiveness of different combinations of tocopherol concentration and diffusivity in the films. In lipid oxidation, poly-unsaturated fatty acid (linoleic acid) is transformed into conjugated dienes which had strong UV absorption at 234 nm, thus lipid oxidation progress can be monitored by measuring the concentration of conjugated dienes. In the early stage of oxidation, the concentration of conjugated dienes kept constantly low, which was indicated by UV showing low value of absorption at 234 nm. This steady state period is commonly known as the induction period and is used as an indicator of shelf life. Once the active oxidation started, the concentration of conjugated dienes increased rapidly, almost linear relationship between time and conjugated dienes concentration. In order to calculate the induction period of lipid oxidation, linear regression was done using the data points after lipid oxidation taking off and the induction period was the intersection of the rapid lipid oxidation line and initial conjugated dienes value at time zero. (Figure 48)
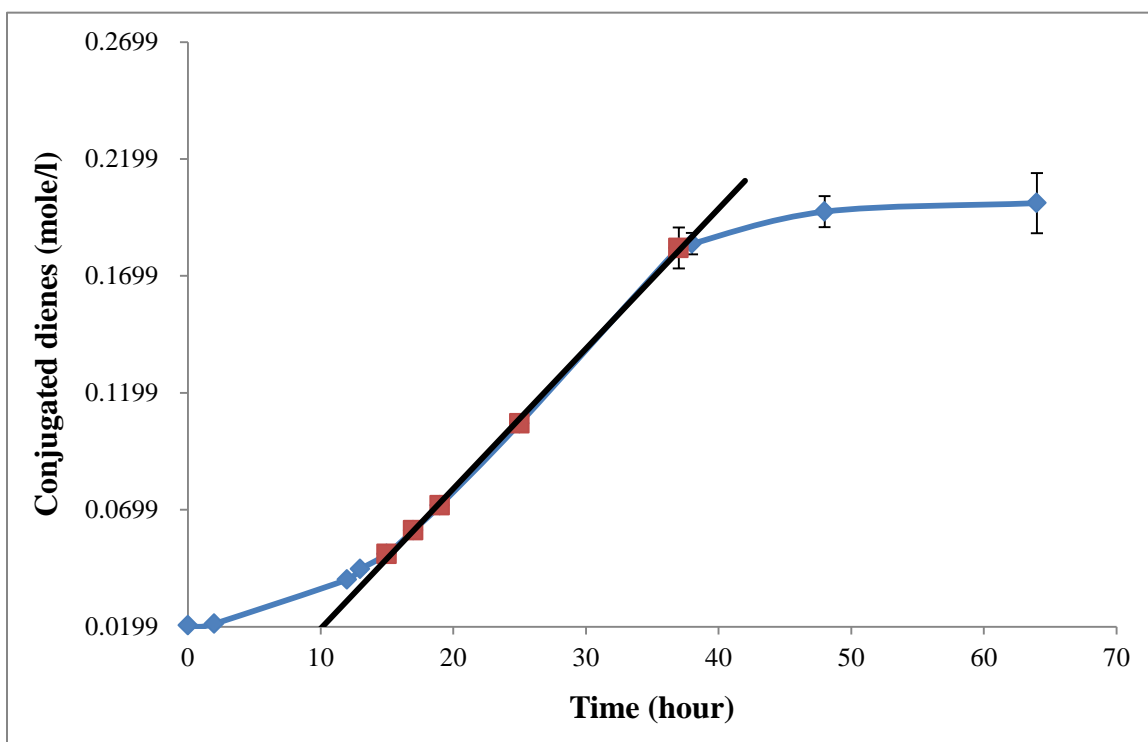
Figure 48: Determination of induction period based on conjugated dienes

5.8.2.   Instant Addition Experiment

In instant addition experiment, 1 ml different tocopherol stock solutions were added into 10 g linoleic acid all at once at time zero, respectively. No more tocopherol solution was added after that. The oxidation kinetics data were shown in Figure 49 at 40 ℃. The concentration of conjugated dienes during oxidation followed the same trend using different tocopherol concentrations: keep low level in the induction period, exponential increase when the oxidation takes off, and decrease in the later stage of oxidation.
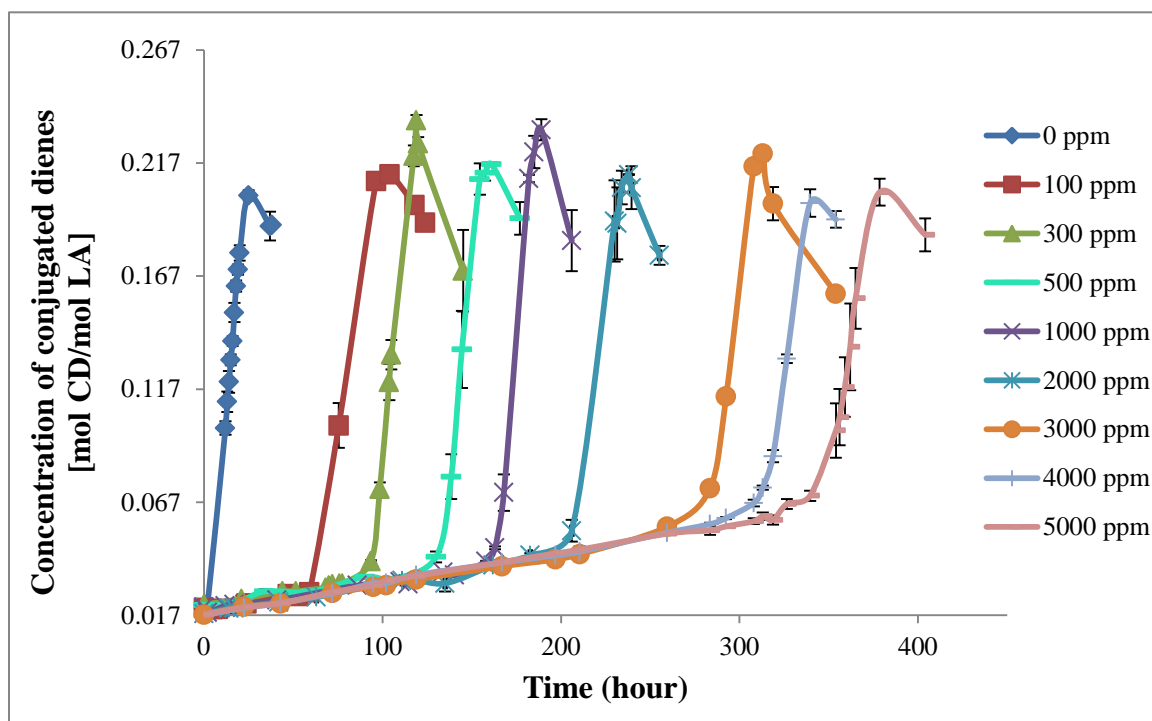
Figure 49: Instant addition of tocopherol in linoleic acid at 40 ℃

The induction period of linoleic acid changed by the concentration of tocopherol, and the results were shown in Table 13. In generally, increasing tocopherol concentration can increase induction period of linoleic acid, and there was no pro-oxidation observed below the concentration of 5000 ppm. However, the relationship between tocopherol concentration and induction period is not linear. For example, 100 ppm tocopherol gave 57 hours of induction period, while three times of tocopherol amount only provides less than two times hours, 91 hours, of induction period. This may be explained that instead of quenching free radicals, some tocopherol radicals formed tocopherol dimers. Thus, tocopherol lost antioxidant effectiveness at higher concentration.

Table 13: Induction period of linoleic acid using different tocopherol concentrations

Concentration of tocopherol (ppm)	Induction Period (hour)
0	4
100	57
300	91
500	130
1000	162
2000	199
3000	274
4000	305
5000	339
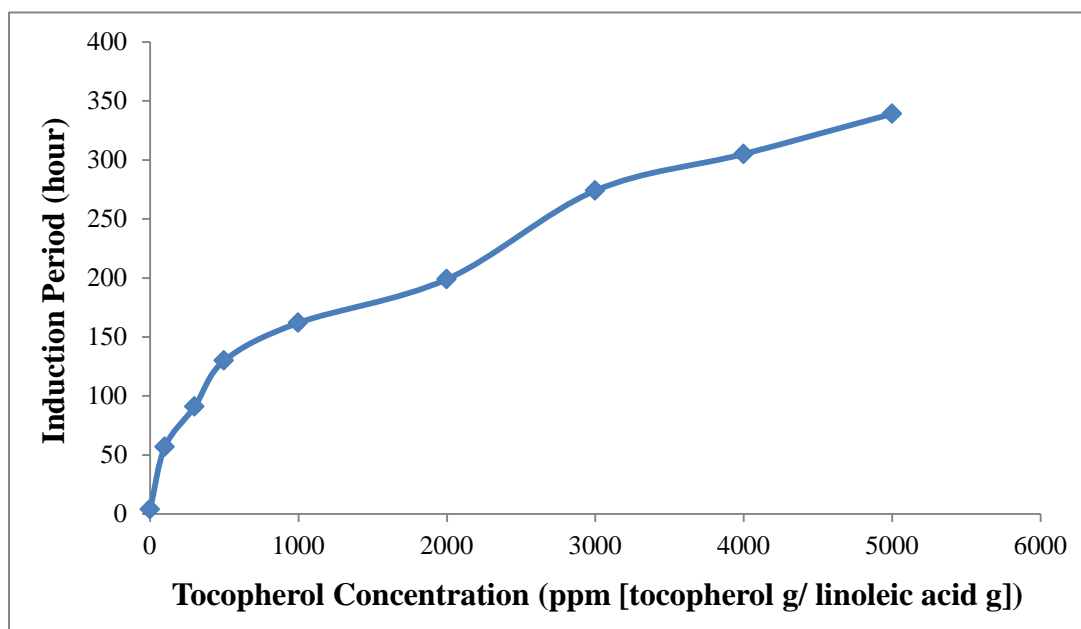
Figure 50: Instant addition of tocopherol in linoleic acid at 40 ℃ plotted using tocopherol concentration

5.8.3.   Syringe Pump Experiment

Lipid oxidation was examined using different delivery modes of tocopherol into linoleic acid by syringe pump system. Instant addition of tocopherol at time 0 was used as the control. For 100 ppm tocopherol, D_{LDPE} (1.03×10^{-13} m^2/s) and $D_{LDPE/PP}$ (3.56×10^{-15} m^2/s) were used. The induction periods of linoleic acid delivered by D_{LDPE}, instant addition and $D_{LDPE/PP}$ were 73, 57, and 47 hours, respectively (Figure 51). Controlled release using D_{LDPE} had longer induction period than instant addition; controlled release using $D_{LDPE/PP}$ had shorter induction period than instant addition. It could be explained that the tocopherol release rate in LDPE/PP blend film was too slow to inhibit lipid oxidation as effectively as instant addition. However, during the induction period, 100% tocopherol amount was delivered into linoleic acid using D_{LDPE} and instant addition, while only 30% tocopherol was delivered into linoleic acid using $D_{LDPE/PP}$.
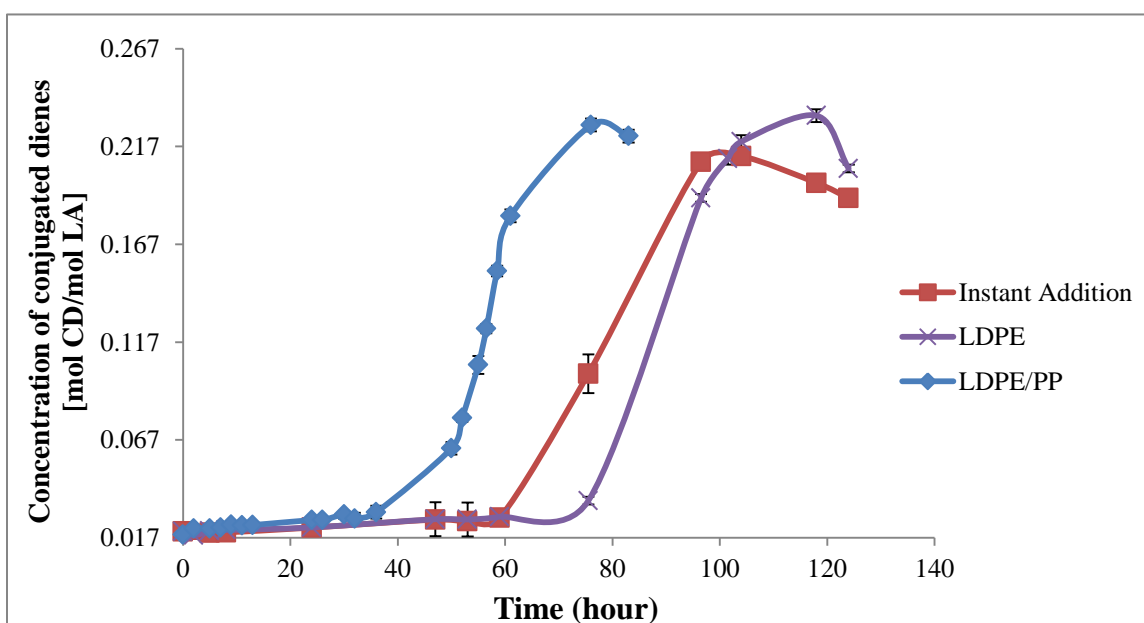
Figure 51: Lipid oxidation kinetics of linoleic acid using 100 ppm tocopherol delivering by D_{LDPE}, $D_{LDPE/PP}$ and instant addition

For 300 ppm tocopherol, D_{LDPE} (1.03×10^{-13} m^2/s) was used. The induction periods of

linoleic acid delivered by D_{LDPE} and instant addition were 213 and 91 hours, respectively

(Figure 52). Controlled release of tocopherol using D_{LDPE} extended the induction period

more than twice of instant addition.
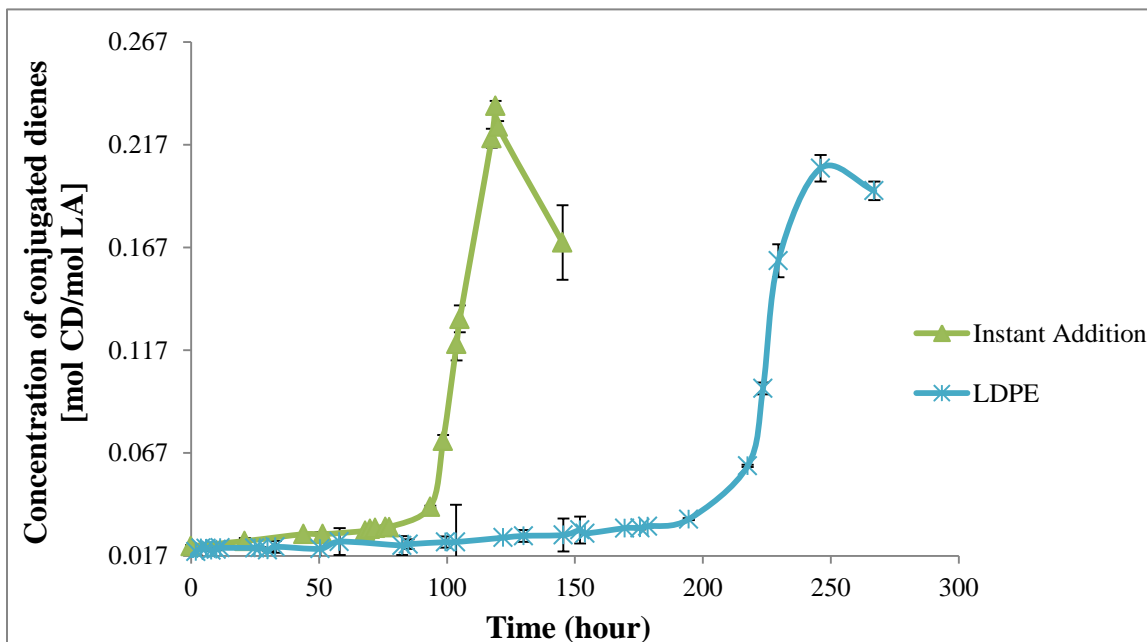
Figure 52: Lipid oxidation kinetics of linoleic acid using 300 ppm tocopherol delivering by D$_{LDPE}$ and instant addition

Compared to instant addition using higher tocopherol concentration, the induction

periods of linoleic acid delivered by D_{LDPE} (300 ppm) was even longer than the result

from instant addition of 2000 ppm (Figure 53). In other words, controlled release of

tocopherol at 300 ppm initial concentration could have the same effect of instant addition

at its six times concentration.
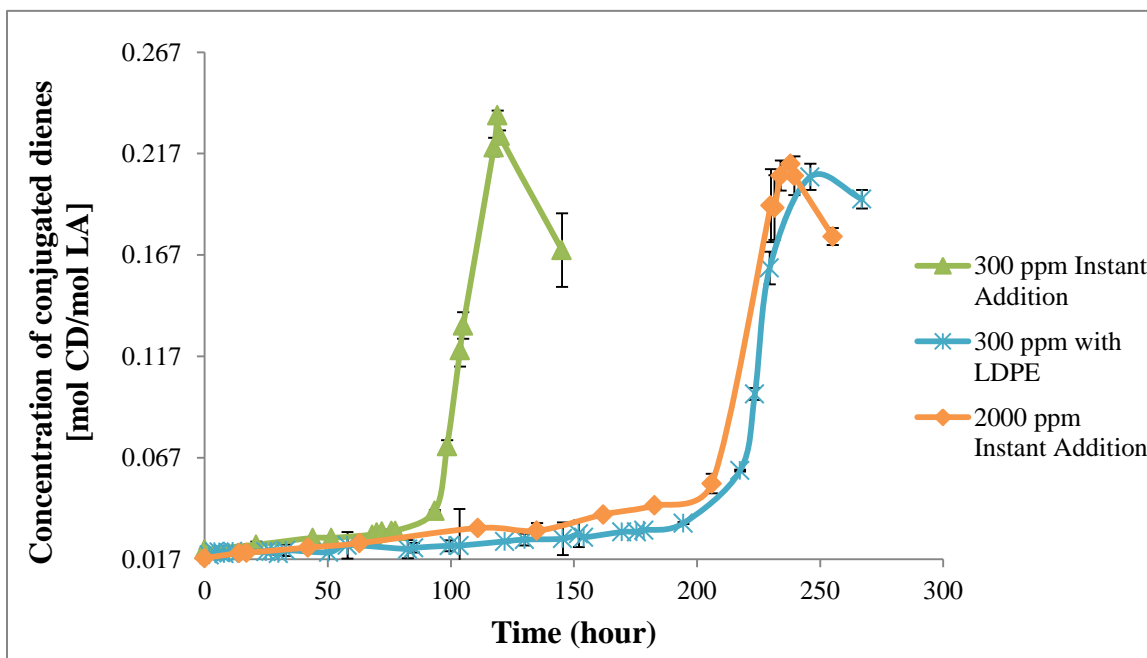
Figure 53: Lipid oxidation kinetics of linoleic acid using 300 ppm tocopherol delivering by D_{LDPE} and instant addition and 2000 ppm instant addition

In summary, induction period of linoleic acid could be extended by controlled release of tocopherol. It may because tocopherol radicals are more than enough in instant addition that they start to form tocopherol dimers, thus lost the antioxidant effectiveness; while controlled release of tocopherol provides appropriate amount of tocopherol into linoleic acid, the induction period is extended under this continuous replenishment. However, too slow release rate may not as effective as instant addition due to insufficient tocopherol to quench free radicals. Tocopherol concentration also effects induction period extension. The extension may not be obvious at low concentration as 100 ppm, but significant extension effect was obtained at high concentration as 300 ppm. CRP could bring shelf life extension effect with much lower concentration of tocopherol compared to instant addition.

The induction period could be extended by controlled releasing less concentration of tocopherol from certain polymer compared to instant addition. For tocopherol-containing LDPE film, at initial concentration of 300 ppm, induction period result from controlled release (D_{LDPE}) was twice of that from instant addition of 300 ppm, and it even had the same effect of instant addition at its six times concentration. Different initial concentrations of tocopherol gave different extension periods. At initial concentration of 100 ppm, induction period result from controlled release (D_{LDPE}) was less than one time of that from instant addition. However, the release of tocopherol from LDPE/PP blend film was not as effective as instant addition because only 30% tocopherol released during induction period, which means that the release of tocopherol was too slow to inhibit the lipid oxidation.

Results show that syringe pump is shown to be able to simulate the release profile from packaging film and inhibit lipid oxidation. Induction period of linoleic acid could be extended by releasing tocopherol in a controlled manner.

6.   CONCLUSION

Release profile of tocopherol from CRP was generated successfully using estimated diffusivity, various concentrations and syringe pump system. The induction period increases significantly with controlled release compared to instant addition. For instant addition, increasing tocopherol concentration 2 times (100→300 ppm) only increased induction period 0.6 times (57→91 hour). For D_{LDPE}, increasing tocopherol concentration 2 times (100→300 ppm) increased induction period 2 times (73→213 hour). It could be explained that the ratio of tocopherol by free radicals was too large for instant addition that tocopherol formed dimmers or trimers, thus losing antioxidant effect. While the delivery mode using D_{LDPE} kept the ratio almost constant during induction period that not too many free tocopherol existed in the system. Most of them may be used to quench the free radicals. Therefore the induction period could be extended much longer than instant addition.

However, not all diffusivities are effective for the same concentration of tocopherol compared to instant addition. For $D_{LDPE/PP}$, induction period is shorter than instant addition when tocopherol concentration was 100 ppm, because only 30% tocopherol was delivered into linoleic acid using $D_{LDPE/PP}$, and this amount is not sufficient to prevent lipid oxidation compared to instant addition. In other word, the ratio of tocopherol by free radicals was too small to stop the chain reaction. If the tocopherol concentration could be increased, the induction period of $D_{LDPE/PP}$ may be extended much longer. There must be

an optimal concentration for each diffusivity value to provide maximal induction period.

All in all, this work confirmed the shelf life extension effect by timed-release of tocopherol. The induction period depends on release profile of tocopherol which is determined by diffusivity of tocopherol in polymer and initial concentration of tocopherol. It demonstrated that controlled release of tocopherol from real CRP films has great impact on induction period extension of lipid oxidation with less concentration of tocopherol, but too slow diffusion of tocopherol in certain polymer may not have the effect to prevent lipid oxidation. This work provides partial quantitative information to help researchers and packaging manufacturers select suitable initial concentration and polymer types based on required shelf life.

7. FUTURE WORK

Since only some combinations of tocopherol concentration and diffusivity values were examined in this research, more combinations could be tested in future. In the later stage of the work, quantifying the optimal concentration of tocopherol incorporated into suitable CRP films (diffusivity of tocopherol in the films) to prolong induction period of lipid oxidation for required shelf life could be done by constructing a profile showing the relationship between induction period, tocopherol concentration and tocopherol diffusivity in polymers. The profile could provide practical information to researchers or packaging manufacturers to design CRP. Other release manner could be invested to compare the current results, such as coating (slow release in the beginning and fast release later).

It is also necessary to do the mass balance of tocopherol in the linoleic acid model system to confirm the results from this research.

Real CRP films packaged with linoleic acid could be investigated to validate the results obtained from syringe pump system. Finally, real food product packaged by CRP films could be examined to compare the differences with food simulants.

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