AVOCADO BYPRODUCT EXTRACT: POSSIBLE USE AS ANTIOXIDANT COATING ON FLEXIBLE PACKAGING

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ABSTRACT

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Food oxidation, as a serious concern of food deterioration, can induce food waste. This spoilage process results from free radical propagation in food molecules and introduces nutrient loss, off-flavor, off-odor and even toxicity issues. Food packaging with an antioxidant coating layer can effectively stabilize free radicals in food products. Avocado byproducts (peel and seed), as reliable and economical sources of natural antioxidants, are rich in phenolic compounds, a predominant group of antioxidants. These food wastes can be utilized for the development of antioxidant packaging. To date, there is no information available in the literature about the applications of avocado byproducts in the packaging field.

The purpose of this research was to extract phenolic compounds from avocado byproducts and to use the crude extracts for the development of an antioxidant coating for three types of packaging films commonly used for food products, i.e., PP, PET and LDPE. To achieve this goal, 70% aqueous ethanol and 70% aqueous acetone were first used to recover phenolic compounds from avocado byproducts. An unconventional extraction procedure was employed to maximize extracted phenolic content within a limited time span. To polymerize the phenolic extracts on the polymer films, a non-metal contact dip coater was developed for this research. Alkaline saline (pH = 7.8) and laccase assist (pH = 5) coating methods were applied. Based on

SEM observations, the coating layer was evenly distributed on the substrates with a thickness of 37.75 ± 0.30 nm; no polymerized clumps were noticed at a high level of resolution.

$AgNO_3$, DPPH•, and ABTS•$^+$ assays were three approaches employed for evaluating the antioxidant efficacy of the phenolic coating in food simulants (95%, 50% and 10% aqueous ethanol). The $AgNO_3$ allowed visual inspection for the existence of phenolic content in the coating layer. The experimental results of DPPH• and ABTS•$^+$ assays showed that the alkaline saline coating technique, an inexpensive approach, could generate a phenolic coating layer with greater antioxidant effectiveness than the laccase assist coating method. Bio-based coating layers with different substrates, extract concentrations, extract ratios (i.e., $W_{peel\ extract}/W_{seed\ extract}$) and coated films with different storage times were tested to analyze antioxidant variation; however, no statistically significant differences were found.

While stabilizing free radicals in food simulants, phenolic compounds in the coating layer did not depend on a migration or surface release process for free radical elimination. Instead, they remained in the coating layer. Presumably, there was more than one layer of phenolic compounds polymerized on the substrates. After donating hydrogen atoms to quench free radicals, phenolic compounds at the surface layer of the antioxidant coating abstracted hydrogen atoms from their adjacent phenolic compounds in an inner layer of the antioxidant coating to continuously serve as antioxidants. It was also noticed that different temperature environments did not impact the stability of the coating layer. All these experimental outcomes implied a promising potential of this bio-based antioxidant coating in future commercial use.

I dedicate this dissertation to my maternal grandmother

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"You can't put a limit on anything. The more you dream, the farther you get." – Michael Phelps.

Pursuing a Ph.D. is a long and tough journey. During the past years, my life was fully filled with tears and joys, ups and downs. Finally, I'm here, standing at the end of the journey. If I were given a second chance, I would still join in the Ph.D. program in the School of Packaging, MSU.

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Jin Zhang

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KEY TO SYMBOLS AND ABBREVIATIONS

AAPH	2,2'-azobis(2-amidino-propane) dihydrochloride
A1	The minimum plateau of a free-radical absorbance reading versus phenolic compound concentration curve
A2	The maximum plateau of a free-radical absorbance reading versus phenolic compound concentration curve
A'_2	The maximum plateau of a free radical quantity versus reaction time curve
ABAP	2,20-azo-bis (2-amidino-propane)hydrochloride
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AgNPs	Silver nanoparticles
ANOVA	Analysis of variance
ANS	Food additives and nutrient sources added to food
Ar : H	An antioxidant
Ar : O•	A phenoxide ion
Ar : OH	A phenolic compound
Ar•	A new free radical generated by the hydrogen atom transferring process of the antioxidant, Ar : H
$Abs_{sample_{t_0}}$	The absorbance reading of DPPH• or ABTS•+ working solutions used for antioxidant test of coated films at time t_0

$Abs_{sample_{t_i}}$	The absorbance reading of DPPH• or ABTS•+ working solutions used for antioxidant test of coated films at time t_i, $i = 1,2 \ldots t$
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DI water	Deionized water
DOPA	3,4-Dihydroxyphenylalanine
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
dx	Time constant
EC50	The effective concentration required for antioxidants to reduce 50% free radicals in the working solution.
EFSA	European Food Safety Authority
EVOH	Ethylene vinyl alcohol
ET50	It refers to the effective time required for the phenolic coating layer to reduce 50% free radicals in a working solution.
FDA	Food and Drug Administration
FRAP	Ferric reducing antioxidant power
Gallol	1,2,3-trihydroxyphenyl
HAT	Hydrogen atom transfer
Hillslope	The slope or steepness of a free-radical absorbance reading versus phenolic compound concentration curve

HS	Headspace
IC	The inhibition capacity of the antioxidant layer. This value is normalized. It varies from 0% to 100%
K2S2O8	Potassium persulfate
LDPE	Low density polyethylene
LSD	Least significant different
Mfps	Mussel foot proteins
•OH	Hydroxyl radical
ORAC	Oxygen radical absorbance capacity
OS	Oxidative stress
PE	Polyethylene
PES	Polyethersulfone
PET	Polyethylene terephthalate
PG	Propyl gallate
PLA	Polylactic acid
Polyphenols	Polymerized phenolic compounds
PP	Biaxially oriented Polypropylene
R•	An alkyl radical
R2	Squared correlation coefficient

ROO : H	A stabilized radical after the hydrogen abstraction reaction
ROO•	An oxygen-derived peroxyl radical of hydrocarbon substrates
ROS	Reactive oxygen species
SEM	Scanning electron microscope
Slope	The steepness of an inhibition capacity curve
t	Logarithm of reaction time
t_0	LogET50
TAC	Total antioxidant capacity
TEAC	Trolox equivalent antioxidant capacity
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble analog of vitamin E
USDA	United States Department of Agriculture
UV-Vis	Ultraviolet-Visible
UHT	Ultra-high temperature
V_{ABTS}	Volume of aqueous ABTS solution
$V_{K_2S_2O_8}$	Volume of aqueous potassium persulfate solution
x	Logarithm of phenolic compound concentration
x_0	The median value of x or LogEC50
y	Absorbance readings of a free radical working solution in a UV-Vis spectrophotometer

y'	Normalized absorbance readings of a free radical working solution in a UV-Vis spectrophotometer. It varies from 0% to 100%

CHAPTER ONE:

Introduction and Objectives

Every year, around 1.3 billion tons of food produced for human consumption are either lost or wasted globally. These food wastes and losses account for roughly one-third of the total production (Gustavsson, Cederberg, and Sonesson 2011). Many factors, such as farming technology, food processing problems, and fluctuations of storage environments, are considered as the causes of this issue. Among all the causal factors, food oxidation is a serious concern. This deterioration process in both aqueous and lipid phases of food products results in the development of nutrient loss, off-flavor, off-odor, color change, and even toxicity of food products. The propagation of free radicals in food molecules is the main reason for the deterioration process. By continuously attacking healthy food cells, free radicals can aggravate their deleterious impact on food products. Under this circumstance, food products will not maintain their acceptable quality until their end consumption. Food manufacturers will thus increase their economic cost for food waste management.

Applying antioxidants to food products is an effective way to retard or prevent oxidation activity and extend product shelf life. While contacting with oxidants in food molecules, antioxidants can serve as chain-breaking electron donors (CB-D). They can donate single electrons or (and) donate hydrogens to deactivate free radicals and thus slow down or stop free

radical propagation. In recent years, the applications of natural antioxidants in active food packaging have drawn remarkable interest from the food industry. Compared with synthetic antioxidants, natural antioxidants are stable, efficient, and more environmentally friendly. Manufacturers normally arouse less concerns if natural antioxidants are employed to protect their food products.

Instead of obtaining natural antioxidants from vegetables, herbs, or fruit pulps, extracting the phytochemicals from food byproducts is a more economical alternative. Based on study results from the fields of food safety and food science, the antioxidant content of some fruit byproducts could be up to 27-fold higher than that of the fruit pulp.

Avocado byproducts are examples of this case. The seed and peel are rich in phenolic compounds, a predominant group of natural antioxidants. Researchers have noticed the potent antioxidant capability of the phenolic extract from different varieties of avocado seeds and peels. To date, no potential health risk or toxic issues of avocado byproducts have been reported. However, there is very limited information available in the literature regarding the applications of avocado byproducts in the food packaging field.

Therefore, the purpose of this research was to extract phenolic compounds from avocado byproducts (seed and peel) and use the extracts to develop an effective antioxidant coating for

three types of packaging films commonly used for food products, i.e., polypropylene (PP), polyethylene terephthalate (PET) and low density polyethylene (LDPE).

For this research, the goals were to:

(1) Develop a method to extract crude phenolic compounds from avocado byproducts (peel and seed).

(2) Develop a non-metal contact coating device.

(3) Coat the phenolic compounds on PP, PET and LDPE films by using alkaline and acidic solutions.

(4) Test and quantify antioxidant activity of the coated polymer films.

(5) Understand the mode of antioxidant activity of the phenolic coating.

In the next chapter of this document, detailed information is provided to explain food oxidation activity, the reaction mechanisms, types, sources, and the applications of antioxidants in the food industry. In addition to avocado byproducts, phenolic compounds and their current applications in the food packaging field are discussed. Chapter three describes the experimental method used in this research for the extraction of phenolic compounds from avocado byproducts. Chapter four describes the development of a non-metal contact coating device together with two different coating solutions. To test the efficacy of the antioxidant coating by using the two different solutions, chapter five presents the analysis and evaluation results of the antioxidant

activities of the coated PP, PET and LDPE films tested in silver nitrate ($AgNO_3$), DPPH• (2,2-diphenyl-1-picrylhydrazyl), and ABTS•$^+$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assays. Chapter six elaborates the mode of antioxidant activity of the biochemical coating layer. In the last chapter, current achievements and further improvement plans are discussed.

CHAPTER TWO:

Literature Review

2.1 Food oxidation

Food oxidation refers to the chemical reaction between food molecules and oxygen. This process happens in both aqueous phases and lipid phases of food products (Skibsted 2010). It not only results in nutrient loss, unhealthy compounds, undesired color change, and unpleasant flavor, but also, it creates free radicals inside food.

These free radicals, as detrimental agents, are classified as reactive oxygen species (ROS). They are highly reactive molecules with an unpaired electron. They can attack most biological molecules at the site of its formation to obtain another electron for stabilization, initiate the propagation of free radical chain reactions, and damage healthy food molecules such as lipids and proteins (Betteridge 2000). As a consequence of both the initial oxidation and the subsequent cascade of reactions, the lifespan of food products can be significantly shortened (Cheeseman and Slater 1993; Lobo et al. 2010; Phaniendra, Jestadi, and Periyasamy 2015; Wasowicz et al. 2004).

Food oxidation is always of great concern. It can occur during manufacture, handling, transportation, storage and preparation processes (Soladoye et al. 2015). It expedites the food deterioration activity, resulting in a soaring number of food products becoming of unacceptable

quality before consumption. Food manufacturers, grocery stores, and consumers, under this circumstance, have to throw away the oxidized food. This contributes to another global issue, i.e., food waste.

In addition, oxidized food products can cause further oxidation reactions during digestion phases of human body, generating other oxidation substances with toxic potential (Van Hecke et al. 2015; Vicente et al. 2012). Via blood distribution and intestinal consumption, the harmful substances may pose risks to internal organs (Estévez and Luna 2017).

Under normal conditions, the self-protection system inside the human body is able to scavenge oxidized products so that the quantities of these harmful compounds are controllable. Once this balance is disturbed oxidative stress (OS) will occur (Estévez et al. 2017). Unmanageable development of OS in crucial molecules, for instance proteins, lipids, and DNA, is normally associated with diseases such as inflammatory bowel diseases, fibrotic degeneration of the liver and kidney, Alzheimer and cataractogenesis (Berlett and Stadtman 1997; Keshavarzian et al. 2003; Li et al. 2014).

2.1.1 Protein oxidation

Protein oxidation prevails in muscle foods. Because of the large and complex structure of protein molecules, free radicals like superoxide anions can easily locate a spot on the molecules to attack, leading to significant modifications of protein conformation, polymerization,

precipitation, etc. (Lund and Baron 2010). This results in food quality degradation; for example meat products with less tenderness (Suman et al. 2014), sharp off-flavors and nutrient loss in dairy products can occur (Citta et al. 2017; Scheidegger et al. 2010).

2.1.2 Lipid oxidation

Lipid oxidation can be found in edible oils, nuts, fatty meat and fish products. At the early stage of lipid oxidation, hydroperoxides can be formed. These vulnerable compounds can be further oxidized and decomposed into acids, alcohols and aldehydes, which are well-known as contributors to the development of nutrient loss, off-odor and off-flavor including undesired rancid taste of food products (St Angelo 1996; Wasowicz et al. 2004). Compared with the undesired changes caused by protein oxidation, lipid oxidation normally leads to more noticeable modifications (Lund and Baron 2010). Lipid oxidation is categorized into three classes, namely autoxidation, photooxidation, and enzymatic oxidation.

2.1.2.1 Autoxidation

Inside the lipid content of food products, oxygen can serve as the trigger of free radical reactions and a substrate for free radical propagation (Porter 1987). This spontaneous degradation process is known as autoxidation. Hydroperoxides are the primary oxidation products of autoxidation. It can further yield other volatile and non-volatile products causing

spoilage and rancidity of food products (Paquette, Kupranycz, and van de Voort 1985; Schultz 1962).

2.1.2.2 Photooxidation

As indicated by the reaction name, this oxidative degradation is triggered by the presence of light, and acts in two ways based on Type I and Type II mechanisms. When a singlet state food photosensitizer like chlorophyll contacts with light, it can be excited by absorbing the light energy. Because of intersystem crossing steps, the excited singlet state photosensitizer can evolve to an excited triplet state photosensitizer. If the excited triplet state photosensitizer reacts with triplet oxygen, singlet oxygen can be generated. This process is called a Type II mechanism. If the excited triplet state photosensitizer abstracts an electron or a hydrogen atom from a substance, radicals can be generated. This process is called a Type I mechanism (Lee 2002; Turro 1985).

2.1.2.3 Enzymatic oxidation

Enzyme catalysts such as cyclooxygenase and lipoxygenase can increase the rate of the oxidation process in the lipid content of food products. They catalyze polyunsaturated fatty acids to generate unsaturated fatty acid hydroperoxides (Henry et al. 2002; Tripathi and Mishra 2016). The occurrence of this reaction creates a pathway for the introduction of toxic compounds in food products (Kubow 1992).

One of the greatest concerns caused by enzymatic oxidation is food product browning. It starts from the oxidation of phenolic compounds in food products, reacts with polyphenol oxidase and other proteins, and finally generates brown pigments, melanosis, on the product surface. This visual deterioration process normally makes food products, especially freshly cut food products, unacceptable (Gonçalves and Oliveira 2016; Jeon, Kim, and Chang 2013; Nirmal et al. 2015).

2.2 Environmental factors influencing food oxidation

Food oxidation is a complex process. Individual environmental factors do not influence food deterioration reactions separately. Rather, in real applications, the oxidation activity is subject to multiple impacts concurrently. Its occurrence and reaction rates are influenced by but not limited to the following environmental factors:

2.2.1 Oxygen

Food oxidation relies on the involvement of oxygen. For lipid oxidation, oxygen paves the way for fatty acid decomposition to develop food rancidity. For protein oxidation, oxygen involved chemical reactions induce amino acid modification, resulting in protein fragmentation or protein-protein cross-linkage. Storing food products in a low oxygen environment has been recognized as an effective method to slow down food oxidation. When lowering the oxygen concentration of the surrounding environment from 21% to 0.5%, researchers noticed significant

decrease in oxygen diffusion in emulsions of fatty substances causing decelerated oxidation activity (Marcuse and Fredriksson 1968). Theoretically, increasing environmental oxygen concentration could ease food oxidation activity. More oxidation products should be detected from food products. However, this was not always the case. Researchers found that within the first 4 days of the experiment, there was no significant difference among the food oxidation product, oxymyoglobin content, of minced beef surrounded by 40, 60, and 80% oxygen in the headspace (O'Grady et al. 2000).

2.2.2 Temperature

Heat provides energy to molecules, and elevated temperatures provide more energy. As the Arrhenius equation implies, molecules with higher energy collide with each other more frequently. Frequent collision leads to higher kinetic energy, which meets the requirement for the activation energy of chemical reactions. The activation energy determines the rate of occurrence of chemical reactions. Therefore, increasing temperature could promote food oxidation reactions. It has been reported that ultra-high temperature (UHT) treatment for dairy product processing could accumulate protein oxidation products such as oxidized amino acid residues causing cross-linked protein species, which could constitute major food allergens (Fenaille et al. 2005). Also, high cooking temperature for meat products could increase the formation of protein carbonyls (Roldan et al. 2014).

2.2.3 Light exposure

Light waves catalyze food oxidation. They influence food oxidation from two aspects: light density and light exposure time. Short light waves with high energy can ease food photooxidation activity (Bekbölet 1990). It was reported that the hydroperoxide content in ice cream, generated by food photooxidation process, could bring about the development of an off-flavor issue (Shiota et al. 2004). Color stability of fresh meat could be disturbed by light-induced myoglobin oxidation (Cooper et al. 2017). After exposing milk products to light, nutrients like vitamin A and riboflavin were degraded and off-flavor was noticed due to the formation of aldehydes in the fat content and the degradation of sulfur-containing amino acids (Brothersen et al. 2016). Light exposure also facilitates oxygen consumption for food oxidation. For cream powders with 35 weeks of storage time, researchers evaluated the product oxidation by measuring the remaining oxygen concentration in the packaging headspace (HS) at each time interval. Compared with the cream powders stored in the dark, the HS oxygen concentration of the cream powders kept in light was significantly lower (Andersson and Lingnert 1998).

2.3 Antioxidants

Antioxidants are chemical compounds which react with free radicals to slow down or inhibit the oxidation activity in food products and thus to extend product lifetimes (Choe and

Min 2009). This defense reaction in food molecules minimizes the negative impact of free radicals on food quality without changing food taste and odor.

2.3.1 Stoichiometric antioxidants

Antioxidants can be either stoichiometric or catalytic reagents. As stoichiometric reagents, antioxidants sacrifice themselves to stabilize free radicals, and thus slow down the product deterioration process. This consumption activity is permanent. When free radicals propagate at a very high rate, stoichiometric antioxidants may not make desired contributions to slow down or stop this detrimental propagation because the total amount of antioxidants is limited (Haber and Gross 2015; Scott 1989).

2.3.2 Catalytic antioxidants

Catalytic antioxidants act differently. They induce antioxidant activity, repeatedly being involved in the free radical stabilization reaction, but they are not consumed. This process is considered as ROS (reactive oxygen species) detoxification activity without self-sacrifice (Golden and Patel 2008). On that account, catalytic antioxidants, even at a low concentration, are still able to present their potent antioxidant ability to inhibit the damaging impacts caused by free radical production (Franck et al. 2013).

2.4 Mechanisms of antioxidant activity

To stabilize free radicals, antioxidants primarily act as kinetic chain-breaking agents. These agents are generally classified into two categories, namely, chain-breaking electron donors (CB-D) and chain-breaking electron acceptors (CB-A).

2.4.1 Chain-breaking electron donors (CB-D)

Scheme 1.1 represents the mechanism of the CB-D reaction. The left part of the equation explains the electron donation process of an antioxidant to a free radical; the right part of the reaction demonstrates the hydrogen abstraction activity of a free radical,

$$ROO^{\bullet} \xrightarrow{+\,e^-} ROO^- \xrightarrow{+\,H^+} ROO : H \qquad \text{(Scheme 1.1)}$$

Where

$ROO^{\bullet}$ is an oxygen-derived peroxyl radical of hydrocarbon substrates

ROO : H is a stabilized radical after the hydrogen abstraction reaction

2.4.1.1 Single electron donation

For the reaction of single electron donation, an antioxidant stabilizes a free radical by donating an electron to the free radical which otherwise could attack and damage healthy molecules. Similar to the hydrogen abstraction reaction, the antioxidant becomes a new free radical after reacting with this free radical. The ionization potential of the antioxidant determines

the occurrence of this reaction. It is also positively correlated to the degree of difficulty of the free radical stabilization process. In other words, the lower the ionization potential of the antioxidant, the easier the single electron donation will be initiated (Ashby 1988; Bendary et al. 2013).

2.4.1.2 Hydrogen abstraction

For the reaction of hydrogen abstraction, antioxidants are used to eliminate peroxidation processes participated in by oxygen-derived peroxyl radicals of hydrocarbon substrates, $ROO^{\bullet}$. During the elimination process, peroxyl radicals abstract hydrogen atoms from antioxidants (Scheme 1.2) to transfer to a more stabilized and less reactive state (Luzhkov 2005; Morello, Shahidi, and Ho 2002). The hydrogen bond dissociation enthalpy influences this antioxidation reaction. It is positively correlated to the degree of difficulty of this free radical stabilization process. In other words, the lower the hydrogen bond dissociation enthalpy in antioxidants, the easier the hydrogen abstraction reaction will take place (Mader, Davidson, and Mayer 2007).

$$ROO^{\bullet} + Ar : H = ROO : H + Ar^{\bullet} \qquad \text{(Scheme 1.2)}$$

Where

$ROO^{\bullet}$ is an oxygen-derived peroxyl radical of hydrocarbon substrates

Ar : H is an antioxidant

ROO : H is a stabilized radical after the hydrogen abstraction reaction

Ar• is a new free radical generated by the hydrogen atom transferring process of the antioxidant, Ar : H

Both the hydrogen abstraction and single electron donation activities are prevalent reactions occurring between antioxidants and free radicals (Morello et al. 2002). They, in most cases, take place concurrently. It is hard to differentiate and identify one reaction from another (Liang and Kitts 2014). In both reactions, the new free radicals are more stable and less reactive compared with the free radicals the antioxidants neutralized, and thus remarkably reduce the risk to unharmed food molecules (Barzegar 2012; Lü et al. 2010; Zhuravlev et al. 2016).

2.4.2 Chain-breaking electron acceptors (CB-A)

Scheme 1.3 represents the mechanism of CB-A reaction. Unlike the CB-D reaction, the free radical in this reaction loses an electron. It basically oxidizes alkyl radicals into non radical products. This reaction efficiency relies on oxygen deficiency of the reaction condition (Al-Malaika et al. 2017).

$$R\bullet \xrightarrow[O_2 \text{ deficient}]{-\,e^-} \text{Non radical product} \qquad \text{(Scheme 1.3)}$$

Where

R• is an alkyl radical

2.5 Synthetic antioxidants and natural antioxidants

There are two types of antioxidants widely used in the food industry, i.e., synthetic antioxidants and natural antioxidants.

2.5.1 Synthetic antioxidants

Synthetic antioxidants are chemical compounds produced by chemical processes. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used synthetic antioxidants to prevent oxidation activity of fats. They are phenolic compounds, and can be applied for butter, snacks, and processed meat products, for example, pork sausage and beef patties. However, it was reported that BHA and BHT have the potential of carcinogenicity (Olsen et al. 1986; Sasaki et al. 2002), and this potential risk to human health has driven researchers to develop different experimental methods to further evaluate the properties of these two synthetic antioxidants (S.-H. Jeong et al. 2005; Vandghanooni et al. 2013).

Propyl gallate (PG), as another type of commonly used synthetic antioxidant, can also be found in the food industry. This phenolic compound is widely applied to meat products, frozen meals, edible oils and soup mixes. Despite the fact that this synthetic compound serves as an effective antioxidant to prevent food rancidity, researchers are unveiling its adverse effects on human health. It has been noticed that the side effects of PG can be skin allergy (García-Melgares et al. 2007), stomach damage, and kidney problems (EFSA Panel on Food Additives

and Nutrient Sources added to Food (ANS), 2014). In the United States, the Food and Drug Administration (FDA) allows the application of PG in the food industry under strict regulations (21 C.F.R. § 582. 3660 (2018)), In European countries, PG is permitted for very limited categories of food products (Annex II of Regulation (EC) No 1333/2008).

2.5.2 Natural antioxidants

Natural antioxidants are phytochemicals. They are natural compounds mainly derived from vegetables, fruits, spices, herbs, and tea leaves. Normally, natural antioxidants are obtained by aqueous extraction processes, safer and more environment-friendly methods compared with the chemical processing methods for obtaining synthetic antioxidants. Considering their antioxidative efficiency, and stability while reacting with oxidants in food products, during recent years, natural antioxidants have drawn increasing attention from the market for food quality protection (Caleja et al. 2017; Carocho et al. 2014; Carocho and Ferreira 2013).

There are three main groups of natural antioxidants: vitamins, carotenoids and phenolic compounds. In order to understand the antioxidative activity of these three categories of antioxidants, researchers evaluated their efficiency in different cultivars. Based on the experimental results of their free radical scavenging ability, phenolic compounds comparatively had greater effect on oxidation prevention (Gil et al. 2002).

Natural ascorbic acid, also known as Vitamin C, is a powerful natural antioxidant (Carocho et al. 2014). It is obtainable in fruits and vegetables. Due to its hydrophilic characteristics, this antioxidant is capable of reacting with free radicals in both lipid and aqueous contents to stabilize food products such as fish, vegetable oils, milk and beverages (Cort, 1982). Since natural ascorbic acid is also served as a nutrient supplement, it has been approved by the FDA as an antioxidant preservative (21 C.F.R. § 145. 110 (2018)).

Lycopene is a well-known natural antioxidant in the category of carotenoids. It is also used as a natural pigment on account of its red color. Tomatoes, guavas, watermelon, and pink grapefruit are rich in lycopene. The food industry extracts this antioxidant from vegetables and fruits and then applies it to beverages, dairy products and sauces to extend product shelf life. Different from BHA and BHT, there is no research revealing that lycopene has the potential of carcinogenicity, nor toxicity (Bánhegyi 2005). On the contrary, researchers presented promising data to indicate that lycopene, other than serving as a strong antioxidant for food products, can be recommended as a dietary supplement as well to improve human health (Paetau et al. 1998; Riccioni et al. 2008; Wood et al. 2008).

2.5.2.1 Natural sources of antioxidants

In addition to vegetables, fruit pulps, spices and herbs, food byproducts from the industry, for instance husks, peels and seeds, also contain antioxidant phytochemicals. Instead of

directly throwing them away to landfills causing environmental impact, an additional value of these non-edible parts can be achieved if they are utilized as a source of natural antioxidants. Compared with vegetables and fruit pulp, using food byproducts is a more economical option. Moreover, experimental results have shown that the antioxidant content of some fruit byproducts could be up to 27-fold higher than that of the fruit pulp (Goulas and Manganaris 2012; Guo et al. 2003; Someya, Yoshiki, and Okubo 2002). The types of natural antioxidants extracted from food byproducts are not limited to a small range. Phenolic compounds, carotenoids and vitamins can all be found in food byproducts (Selvamuthukumaran and Shi 2017). Among them, phenolic compounds with high antioxidant potency are predominant (Moure et al. 2001). Due to the aforementioned facts, increasing numbers of researchers have started using food byproducts as attractive sources for natural antioxidant experiments (Table 2.1).

Table 2.1 Examples of natural antioxidants extracted from food byproducts.

Antioxidant Source	Types of Antioxidants	References
Pomegranate peel and seed	Phenolic compounds	Singh et al. (2002)
Start fruit residue	Phenolic compounds	Shui et al. (2006)
Grape waste	Phenolic compounds	Lafka et al. (2007)
Citrus peel	Phenolic compounds	Xu et al. (2008)
Mango peel powder	Phenolic compounds and carotenoids	Ajila et al. (2010)
Avocado seeds and peels	Phenolic compounds	Kosińska et al. (2012)
Coffee spent grounds and silverskin	Phenolic compounds	Jiménez-Zamora et al. (2015)
Tomato peel	Lycopene and phenolic compounds	Elbadrawy et al. (2016)
Orange, lemon and grapefruit wastes	Vitamin C and phenolic compounds	Sir Elkhatim et al. (2018)

2.5.2.2 Crude extraction methods for natural antioxidants

To utilize antioxidants from natural sources, crude extraction is the first step. This process is primarily influenced by solvent type, concentration and polarity, extraction duration and temperature, pH value of the extraction solution, and solubility of natural antioxidants in the extraction solvent.

A wide range of crude extraction solvents have been reported for natural antioxidants. For water-soluble antioxidants, for instance ascorbic acid, phenolic compounds, flavonoids and glutathione, pure or aqueous solvents with high to medium polarity were used for the extraction. Common solvents for water-soluble antioxidants are water, ethanol, methanol and acetone (Abu et al. 2017; Boeing et al. 2014). For lipid-soluble antioxidants, for example vitamin A, vitamin E, and carotenoids, organic solvents with medium to low polarity, such as ethanol, ether and benzene, were reported for the extraction (Ghasemzadeh et al. 2015; Traber and Atkinson 2007).

Crude extraction procedures can be categorized into two groups, i.e., conventional and unconventional. Hot water bath, maceration and Soxhlet extraction method are classified as conventional extraction approaches. They are regarded as time-consuming and costly means with low yield of efficiency (Selvamuthukumaran and Shi 2017; Zhang, Lin, and Ye 2018). In contrast, unconventional extraction procedures like ultrasound, microwave, pressurized, pulsed

electric field and enzyme hydrolysis are proposed due to their economic, environmental friendly and high efficiency attributes (Hidalgo and Almajano 2017; Xu et al. 2017).

2.6 Most common assays for antioxidant evaluations

To evaluate free radical elimination ability of antioxidants, researchers have developed various experimental methods. The free radical decreasing process can be quantified by measuring a free radical absorbance peak with a UV-Vis spectrophotometer at a particular wavelength. For some of the testing approaches, the free radical elimination process can also be visualized by observing color change of the free radical working solutions.

2.6.1 ORAC assay

ORAC stands for oxygen radical absorbance capacity. This assay is utilized for testing the antioxidant efficiency of hydrophilic antioxidants (Huang et al. 2002).

In ORAC testing, natural antioxidants slow down the degradation rate of fluorescent molecules (normally fluorescein) due to the attack from a free radical generator, such as AAPH ((2,2'-azobis(2-amidino-propane) dihydrochloride). To reduce free radical attack, natural antioxidants transfer their hydrogen atoms (HAT) to stabilize free radicals. This transfer efficiency is quantified by creating decay curves of fluorescent molecules, compared with standard decay curves, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a

water-soluble analog of vitamin E) curves, and expressed as Trolox equivalence (Kohri et al. 2009; Roy et al. 2010).

2.6.2 FRAP assay

FRAP stands for ferric reducing antioxidant power. It determines the antioxidant ability of phytochemicals by measuring the reduction volume of ferric ions. At low pH value (3.6), ferric ions (Fe^{3+}) in working solution will be gradually transformed to ferrous ions (Fe^{2+}). The transformation process can be visualized by a blue color development of the working solution, and quantified by measuring the absorbance peak at 593 nm with a UV-Vis spectrophotometer (Benzie and Strain 1996).

The FRAP assay is easy to prepare. It does not require a complicated detection method to evaluate the rapid transformation process. However, the low pH value of the working solution does not represent physiological conditions (López-Alarcón and Denicola 2013).

2.6.3 TEAC assay

Trolox equivalent antioxidant capacity (TEAC) assay was developed for total antioxidant capacity (TAC) determination (Miller et al. 1993). This method was later modified by Van den Berg et al. (1999).

According to Van de Berg et al., fresh radical anions of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) working solution, $ABTS^{\bullet -}$, need to be prepared every

time for the antioxidant testing. It can be obtained by mixing ABAP (2,20-azo-bis (2-amidino-propane)hydrochloride) with ABTS$\bullet^{2-}$ stock solution. After adding natural antioxidants to the working solution, the antioxidant efficiency can be analyzed by measuring the mixture at 734 nm with a UV-Vis spectrophotometer for 6 minutes. The testing result is expressed as Trolox equivalents by comparing it with Trolox standard graphs.

To minimize environmental interference, light exposure should be avoided throughout the test.

2.6.4 DPPH$\bullet$ and ABTS$\bullet^{+}$ assays

DPPH$\bullet$ (2,2-diphenyl-1-picrylhydrazyl) and ABTS$\bullet^{+}$ are stable free radicals. Their working solutions are purple and blue-green in color, respectively.

To conduct the antioxidant test, ABTS$\bullet^{+}$ needs to be prepared by oxidizing ABTS with $K_2S_2O_8$, whereas DPPH$\bullet$ can be directly purchased. During the test, antioxidants stabilize these radicals resulting in free radical reduction. This radical concentration decreasing process can be measured with a UV-Vis spectrophotometer at 517 nm (DPPH$\bullet$) or 734 nm (ABTS$\bullet^{+}$).

For this research, DPPH$\bullet$ and ABTS$\bullet^{+}$ assays were selected to evaluate the antioxidant efficacy of the phenolic coating layer on PP, PET and LDPE films. More discussions regarding these testing methods are included in chapter five.

2.7 Antioxidant applications in the food industry

Synthetic and natural antioxidants have two main applications in the food industry: either directly introduced to food products as additives or applied to food packaging to maintain product smell, color, taste, and texture until final consumption.

2.7.1 Food additives

Food additives can be introduced to food products at any step of their processing and production procedures. Manufacturers, traditionally, add BHA, BHT and PG into edible oils to ensure product quality (Raikos 2017), and ascorbic acid and tocopherols into milk products to prevent milk protein oxidation (L.H. Skibsted 2010). The introduced volume of different additives is under strict control by regulatory authorities. Food products in European countries with antioxidant additives must comply with European Food Safety Authority (EFSA) regulations. For food products manufactured and consumed in the United States, they must follow food safety regulations issued by the Food and Drug Administration of the United States of America (FDA) and United States Department of Agriculture (USDA). The allowed content of each antioxidant is not invariable. Based on researchers' investigation, study and experimental results, the authorities inspect and re-evaluate potential safety risks of each antioxidant and revise the corresponding regulations regularly.

2.7.2 Antioxidant active packaging

When antioxidants were employed in the packaging field, researchers developed antioxidant packaging. Antioxidant packaging is classified as active packaging. It is widespread in the food, pharmaceutical and cosmetic industries due to their potent ability to prolong product shelf life.

2.7.2.1 Active packaging

In order to obtain desired surrounding conditions for a product, active packaging applies different technologies to adjust the inside environment of a sealed packaging system. Depending on the type of the packaged product, the applied technologies may vary. During transportation and delivery, even faced with the fluctuations of temperature and relative humidity, the packaged product, under the effective protection from its active packaging, can still be prevented from spoilage, off-flavor, and off-odor issues, and finally present its high quality to end consumers. Thanks to active packaging, manufacturers, especially food manufacturers, each year significantly reduce their expenses for handling products with unacceptable quality. The environment thus can suffer less impact.

The following table includes active packaging types that are prevailing in the food industry (Coles, McDowell, and Kirwan 2003; Ozdemir and Floros 2004; Patel 2018; Prasad and Kochhar 2014; Yildirim et al. 2018).

Table 2.2 Examples of prevalent active packaging in the food industry.

Packaging Type	Mechanisms	Packaging Application
Oxygen scavenger	Use oxygen absorbing sachets inside packaging to reduce excessive oxygen in packaging headspace	Cheese spread, cakes, chocolate, vegetable juice, etc.
Ethylene scavenger	Use ethylene absorbing sachets inside packaging to reduce excessive ethylene in packaging headspace	Climacteric fruits and vegetables, etc.
Carbon dioxide controlled	Use carbon dioxide releasing or absorbing sachets inside packaging to regulate gas composition in packaging headspace	Fresh fish and meat products, etc.
Odor removal	In order to remove odoriferous byproducts in packaging headspace, a. Use sachets filled with active carbon, b. Incorporate antioxidants or other odor removers in packaging substrates, or c. Apply an antioxidant coating onto packaging substrates	Wine, peanut butter, etc.
Humidity-controlled	In order to maintain the product quality of moisture sensitive products, use a. Desiccant sachets, b. Packaging systems with humidity regulators, or c. Packaging materials with selective gas permeability	Mushroom, tomato, strawberries, etc.

Table 2.2 (cont'd)

Temperature-controlled	In order to maintain the product quality of temperature sensitive products, Use a. Self-cooling cans b. Special insulating materials c. Dry ice packs d. Self-heating packaging	Seafood, sake, coffee, tea, ready meals, etc.
Antimicrobial	In order to prevent microbial growth in food products, a. Incorporate antimicrobial agents in packaging substrates, or b. Apply an antimicrobial coating onto packaging substrates	Fresh and processed meat, fresh seafood, dairy products, etc.
Antioxidant	In order to slow down food deterioration process and prevent food rancidity, a. Incorporate antioxidants in packaging substrates, or b. Apply an antioxidant coating onto packaging substrates	Seed, nuts, edible oil, fried products, fresh vegetables and fruits, etc.

2.7.2.2 Antioxidant active packaging

Compared with directly adding antioxidants in food products, incorporating antioxidants in packaging substrates or applying antioxidant coatings onto packaging substrates may use a smaller amount of antioxidants. When a packaging substrate starts releasing antioxidants to its food product, or an antioxidant coating is contacting with its packaged food product, the

antioxidant activity is initiated. To retard the deterioration process of the food products, free radicals in the food cells are scavenged by antioxidants to stop further deleterious chemical reactions. The potency of the antioxidant activity depends on various aspects, including the release rate of the antioxidants in the packaging substrate, the density of the antioxidant coating, the mechanisms of the antioxidants reacting with different types of free radicals, the water content of the food products, the lipid content of the food products, and environmental factors.

Table 2.3 lists some examples of recent research on antioxidant packaging that can be applied for food products.

Table 2.3 Selective examples of antioxidant packaging research for food products since 2010.

Packaging Substrate	**Antioxidant(s)**	**Packaging Technology**	**Tested Food Products**	**Experimental Results**	**Reference**
Poly (lactic acid) film	α-Tocopherol	Twin screw extrusion (Antioxidant incorporation)	Soybean oil	Retarded the oxidation process of soybean oil	Manzanarez-López et al. (2011)
EVOH film	Natural flavonoids (quercetin and catechin)	Twin screw extrusion (Antioxidant incorporation)	Fried peanuts and sunflower oil	The flavonoids effectively reduced the radical oxidative species presented in both food products	López de-Dicastillo et al. (2011)

Table 2.3 (cont'd)

Chitosan film	Green tea extract	Solvent casting (Antioxidant incorporation)	Pork sausages	The green tea extract enhanced the antioxidant and antimicrobial properties of the chitosan film	Siripatrawan et al. (2012)
LDPE film	Tocopherol mixture	Twin screw extrusion (Antioxidant incorporation)	Salmon (*Salmo salar*)	Effectively conserved the salmon samples for long-term storage	Barbosa-Pereira et al. (2013)
LDPE film	Natural phenolic compounds from brewery residual and rosemary extract	Antioxidant coating	Beef	The coated film enhanced the oxidative stability of the meat product	Barbosa-Pereira et al. (2014)
PET/PE/ EVOH/PE film	Natural oregano essential oil or solid green tea extract	Antioxidant coating	Foal meat	The antioxidant coating film containing oregano essential oil presented better protection to retard oxidation, retained product color and odor	Lorenzo et al. (2014)

Table 2.3 (cont'd)

LDPE film	Murta leaf extract in a methylcellulose layer	Antioxidant coating	Milk chocolate	The antioxidant and antimicrobial effectiveness of the coated film could last for 60 days. Plus, food off-flavor and off-odor issues were not noticed	Hauser et al. (2016)
Raw paper sheets	Citric acid in gelatin	Antioxidant coating	Beef	After four days of storage, the meat product had a lower microbial population, better oxidation stability, and presented desired red color	Battisti et al. (2017)

Table 2.3 (cont'd)

PE film	Green tea extract	Antioxidant encapsulation in extruded PE film	Minced pork meat	The antioxidant packaging extended the meat product shelf life for 3 days and preserved the red color of the pork meat	Wrona et al. (2017)
Oriented PP film	Clove essential oil or eugenol	Antioxidant coating	Beef	During the 14 days of storage time, the antioxidant coating layer reduced the formation of oxidative compounds and protected the color of the meat product	Navikaite-Snipaitiene et al. (2018)

Table 2.3 (cont'd)

Bio-based (Polylactic acid) emitting sachets	Eugenol, carvacrol, and trans-anethole	Inserted the sachets into cellulose and PP pillow	Fresh-cut iceberg lettuce	Eugenol and trans-anethole reduced discoloration of the lettuce, preserved sensory quality of the lettuce, and introduced formation and accumulation of phenolic compounds inside the packaging	Wieczyńska et al. (2018)
PET/LDPE film	Sage leaf or bay leaf extracts	Added the antioxidants in the film adhesive	Fried potatoes	The antioxidant film effectively retarded the food oxidation process and decreased oxidative products	Oudjedi et al. (2019)

2.8 Avocado

2.8.1 Avocado and its byproducts

Avocado (Persea americana Mill.) is one of the most common fruits in the United States, originating from Central Mexico. Among the hundreds of varieties of avocados, Fuerte and Hass are the most common varieties in the food market. Because of the high content of bioactive

compounds (including vitamin C, vitamin E, carotenoids and phenolic compounds (Antasionas, Riyanto, and Rohman 2017)) in its fruit pulp, avocado has been recognized as a functional fruit providing great benefits for human health. Based on clinical study results, consuming avocados aided the healthy aging process, benefited cardiovascular health, and helped cholesterol level management in the human body (Dreher and Davenport 2013).

Nowadays, avocado, a highly nutritious fruit, has become widely known around the world. Its production has spread from Mexico to the United States, Australia, South Africa, and Spain (Rodríguez-Carpena et al. 2011). From 1994 to 2004, the global production volume of avocado dramatically soared from 4.6 billion pounds to 6.8 billion pounds. The United States is ranked as the second largest avocado producer in the world, following Mexico. The fruit growers can be found in California, Florida and Hawaii. Every year, California alone is estimated to produce 400 million pounds of avocados (Dreistadt 2007). These huge numbers imply a large quantity of avocado waste could be generated every year, including spoiled avocado, and avocado seed and peel left by human consumption and the food processing industry. Thus, handling the fruit waste requires substantial costs for food waste management. If additional value of the food waste could be added to avocado, the food industry would show further interest in this crop. This would also bring economic benefit to the food growers and manufacturers.

2.8.2 Additional value of avocado byproducts

Avocado seed and peel are ideal sources of natural phenolic compounds, which belong to the major group of natural antioxidants. According to study results from food safety and food science fields, the peels and seeds of Fuerte and Hass avocados contain significantly higher phenolic contents than the avocado pulps, and the phenolic extract from these avocado byproducts presented robust antioxidant capability to inhibit oxidation reactions. (Rodríguez-Carpena et al. 2011; Soong and Barlow 2004; Torres, Mau-Lastovicka, and Rezaaiyan 1987; Wang, Bostic, and Gu 2010).

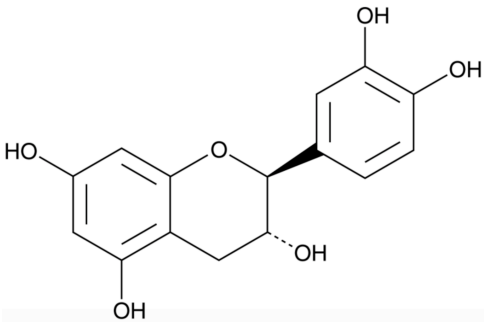
Avocado seed and peel are rich in a mixture of phenolic compounds. The prevalent natural phenolic compounds, such as catechin, epicatechin gallate, procyanidin, chlorogenic acid, protocatechuic acid, syringic acid, rutin, and quercetin, all exist in avocado seed and peel (Kosińska et al. 2012; Pahua-Ramos et al. 2012; Tremocoldi et al. 2018). Table 2.4 and Figure 2.1 present primary phenolic compounds and their contents in Hass avocado byproducts. The phenolic content was determined based on dry weight (DW). In addition to the antioxidant property, avocado byproducts also have anti-inflammatory and analgesic properties (Kristanti et al. 2017; Tremocoldi et al. 2018). These great potentials have aroused interest from the pharmaceutical industry. Researchers can use them as reliable sources to prevent inflammatory diseases.

Table 2.4 Primary phenolic compounds and their contents in Hass avocado byproducts as reported in literature (Kosińska et al. 2012; Pahua-Ramos et al. 2012; Tremocoldi et al. 2018).

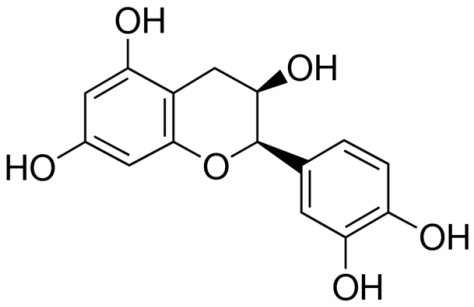
Compound Name	Type of Byproduct	Content (μg/g DW)	
		Peel	Seed
Catechin	Peel & Seed	148.80 ± 5.95	152.80 ± 14.60 (with epicatechin gallate)
Epicatechin	Peel & Seed	40.21 ± 0.24	10.27 ± 0.08
Procyanidin dimer B (I)	Peel	135.40 ± 7.44	N/A
5-O-caffeoylquinic acid	Peel	81.80 ± 5.95	N/A
Procyanidin dimer B (II)	Peel	55.10 ± 4.46	N/A
Quercetin 3-O-galactoside	Peel	31.20 ± 4.46	N/A
Quercetin-3-O-rutinoside (rutin)	Peel	23.80 ± 2.98	N/A
Protocatechuic acid	Seed	N/A	128.18 ± 0.01
Kaempferide	Seed	N/A	107.42 ± 0.04
Procyanidin trimer A (II)	Seed	N/A	89.30 ± 9.73

Table 2.4 (cont'd)

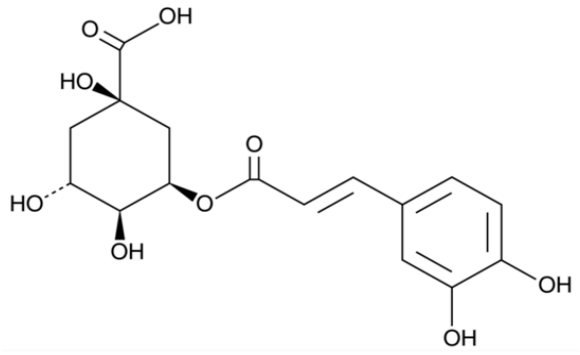
Procyanidin trimer A (I)	Seed	N/A	81.70 ± 6.49
3-O-Caffeoylquinic acid	Seed	N/A	57.50 ± 6.49
Vanillic acid	Seed	N/A	28.67 ± 0.001



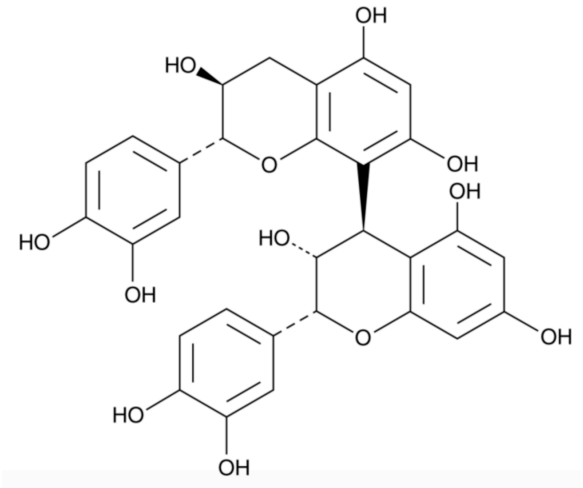
Catechin



Epicatechin



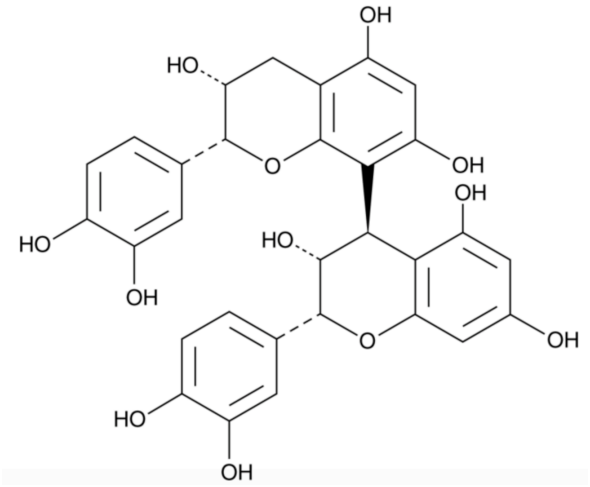
5-O-caffeoylquinic acid



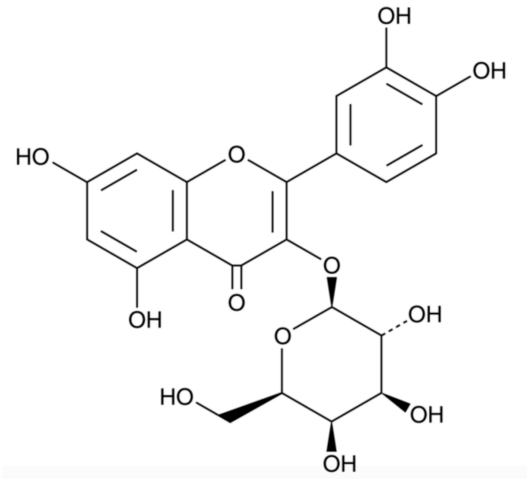
Procyanidin dimer B (I)

Figure 2.1 Chemical structure of primary phenolic compounds in Hass avocado byproducts.

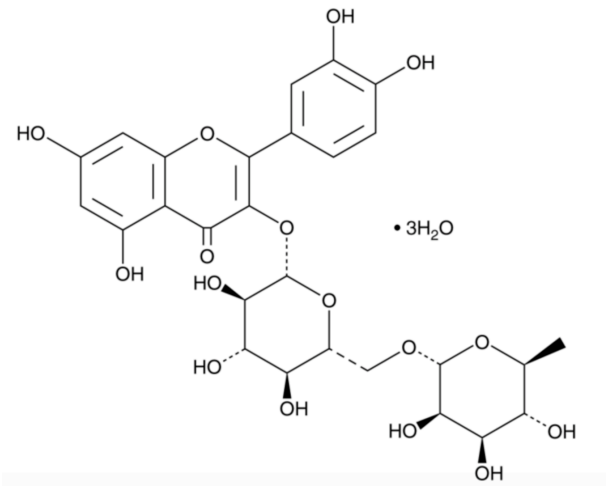
Figure 2.1 (cont'd)



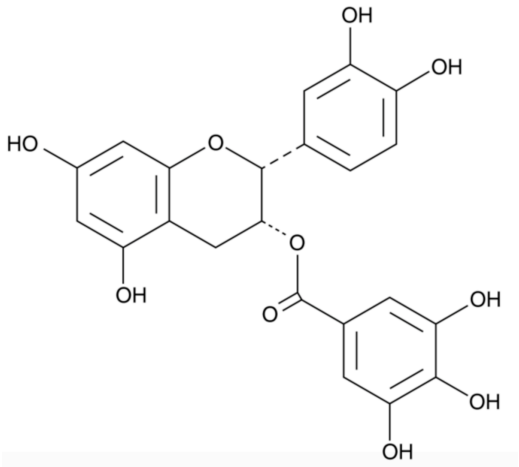
Procyanidin dimer B (II)



Quercetin 3-O-galactoside

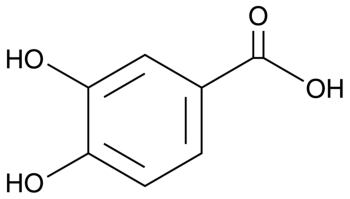


Quercetin-3-O-rutinoside (rutin)

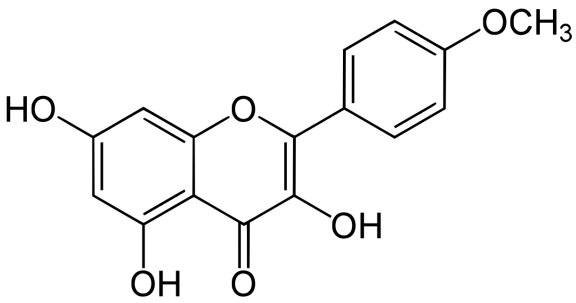


Epicatechin gallate

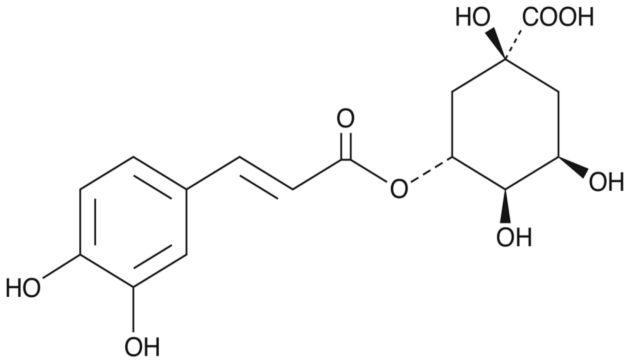
Figure 2.1 (cont'd)



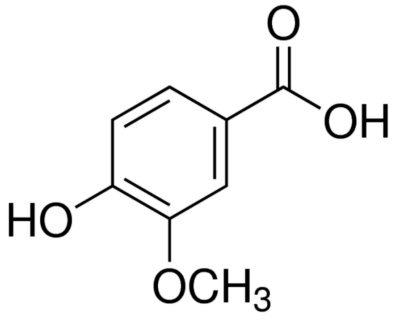
Protocatechuic Acid



Kaempferide



3-O-Caffeoylquinic acid



Vanillic acid

2.8.3 Applications of avocado byproducts in the packaging field

To date, there is no information available in the literature discussing applications of avocado byproducts in the packaging field. However, in 2012, a Mexican company named Biofase claimed that they developed a biodegradable plastic by using avocado waste (Anon 2012). According to the chemical engineer and founder of Biofase, around 30,000 tons of avocado seeds are discarded by the Mexican industry each month. In most cases, these avocado seeds were directly burned at landfill sites. The biomaterial offered an environmentally friendly solution to improve the situation. No further information was provided to show that this technology had been put into production. Experimental data in terms of the chemical and physical properties of the biomaterial were missing.

2.9 Phenolic compounds

Phenolic compounds refer to chemical compounds with one or more hydroxyl groups attached to a carbon atom of an aromatic ring. A phenolic compound should have at least one aromatic ring. There are more than 8000 types of phenolic compounds with different chemical structures that have been identified (Martínez-Valverde, Periago, and Ros 2000). They vary from a single-aromatic ringed chemical with low molecular weight to a complicated polyphenol with multiple aromatic rings. Based on the number of aromatic rings and the carbon atom arrangement, phenolic compounds can be grouped into 9 categories. They are phenolic acids,

acetophenones, phenylacetic acid, hydroxycinnamic acids, coumarins, naphthoquinones, xanthones, stilbenes, and flavonoids (Crozier, Jaganath, and Clifford 2007).

Phenolic compounds utilize their redox properties to serve as antioxidants. While reacting with oxidative chemicals, they can donate electrons, transfer hydrogens, and chelate metal ions such as iron and copper (Bendary et al. 2013; Estévez et al. 2008; Zhuravlev et al. 2016). These different reaction mechanisms allow phenolic compounds to exhibit strong antioxidant capability.

2.9.1 Applications of natural phenolic compounds in the packaging field

Because of the remarkable interest in the applications of natural antioxidants from the food industry, natural phenolic compounds, as the predominant phytochemicals, have been evaluated by a variety of packaging researchers. Based on the experimental results, the biochemicals exhibit robust antioxidant capability to scavenge free radicals in tested samples, slow down or prevent the browning process of food products, and maintain food taste and texture. In view of the stability and solubility of natural phenolic compounds, researchers recommended natural phenolic compounds as trustworthy biochemicals to protect food products from oxidation and rancidity.

Natural phenolic compounds are easy to obtain. Extracts from many herbs, spices, vegetables, and fruits contain phenolic compounds. Määttä-Riihinen et al. (2004) identified and

quantified the phenolic compounds in berries. Rusak et al. (2008) and Yang et al. (2012) evaluated the phenolic contents of different types of tea plants. Roby et al. (2013) tested the total phenolic compounds in thyme, sage, and marjoram. Cheng et al. (2013) examined the antioxidant efficacy of phenolic compounds in red and yellow onions.

There is a broad range of food products that can be protected by natural phenolic compounds. The phytochemicals can be utilized for red meat, fish, dairy products, beverages, edible oils, etc. (L. H. Skibsted 2010). Table 2.5 lists several examples of the applications of natural phenolic compounds in food packaging.

Table 2.5 Examples of applications of natural phenolic compounds in food packaging.

Phenolic Sources	Packaging Type	Food Application	Reference
Grapefruit extract	A bio-based film layer containing the antioxidants	Pork loins	Hong et al. (2009)
Barley husk extract	Antioxidant coating on PE film	Atlantic salmon (Salmo salar L.)	Pereira de Abreu et al. (2010)
Green tea extract	Packaging film containing antioxidants	All type of foods, from aqueous to fatty products	López de Dicastillo et al. (2011)
Grape seed extract	Modified atmosphere packaging	Pork patties	Kumar et al. (2015)
Oregano extract	Antioxidant coating on PP film	Beefsteak	Djenane et al. (2016)

CHAPTER THREE:

Crude Extraction of Phenolic Compounds from Avocado Byproducts

3.1 Introduction

For the crude extraction of phenolic compounds from food or food byproducts, researchers commonly utilize methanol, ethanol, acetone, and ethyl acetate as the solvents. Both pure and aqueous mixtures (DI water and solvent mixture) can be used. Normally, 70% aqueous mixtures are used for extraction to recover phenolic compounds with desired antioxidant efficacy from food or food byproducts. Solvent polarity is one key factor that determines the concentration and antioxidant efficacy of phenolic extracts from food or food byproducts (Antasionas et al. 2017; Naczk and Shahidi 2006). In addition, the solubility of extracted phenolic compounds in solvents also affects the phenolic content recovered from food or food byproducts (Alothman, Bhat, and Karim 2009).

While extracting phenolic compounds from food or food byproducts, temperature is another important aspect that needs to be considered. Moderate heat could assist the extraction, and thus increase phenolic content. However, when the temperature is elevated to a certain level, decomposition of phenolic compounds may be introduced, causing the reverse effect (Liyana-Pathirana and Shahidi 2005; Tan, Tan, and Ho 2013).

The experimental conditions for phenolic extraction from different food or food byproducts are not universal. In addition to solvent polarity, the solubility and polarity of extracted phenolic compounds and extraction time are other critical factors that need to be considered. Based on the literature, extraction time varied based on the types of food and food byproducts. Alothman et al. (2009) used 3 hours to obtain phenolic compounds from honey pineapple (*Ananas comosus* Merr.), banana (*Musa paradasiaca*) and guava (*Psidium guajava* L.). Lafka et al. (2007) changed the extraction time from 30 minutes to 24 hours to obtain phenolic content from winery wastes. Ajila et al. (2010) used 15 minutes to complete the extraction process. To extract phenolic compounds from avocado byproducts, Folasade et al. (2016) used 48 hours to allow the solvent to completely react with avocado seed powder. Calderón-Oliver et al. (2016) shortened the time to 30 minutes to extract phenolic contents from avocado seed and peel powders.

Due to the acceptability for human consumption and the solubility of a wide range of phenolic compounds, ethanol was selected as one solvent for the phenolic extraction from avocado byproducts. Moreover, to understand the influence of different solvents with different polarities on the antioxidant effectiveness of coated films, acetone, as a most efficient solvent for phenolic extraction reported by Alothman et al. (2009) and Folasade et al. (2016), was also

selected for this research. Trial-and-error was used to determine the byproduct quantity, extraction time, and solvent temperature for the extraction process of this research.

3.2 Materials and methods

3.2.1 Byproducts preparation

Raw avocado seed powder was purchased from Addicted 2 Healthy Nutritional SuperFoods, LLC. The powder was stored under refrigeration at 4 °C until use.

Fresh Hass avocado (product of Mexico) peels were supplied by local sushi restaurants. They were first cleaned using tap water to remove dust particles and other contaminants on the peel surface, and air dried at room temperature for 12 hours. To completely remove the moisture content, the avocado peels were then put in a freeze dryer for another 12 hours. Afterwards, the peels were ground into small particles using an Eberbach E3300 mill (Belleville, MI, U.S.A.) with a number 40 mesh sieve. The peel particles passing through the sieve were around 0.42 mm in diameter. Finally, the ground peels were stored in plastic jars with screw-on lids under refrigeration at 4 °C until use. In order to control the relative humidity surrounding the ground peels, the plastic jars were placed inside Ziploc bags together with desiccant sachets.

3.2.2 Extraction of phenolic compounds

3.2.2.1 Materials

Ethanol (200 proof) was purchased from VWR International (Radnor, PA, U.S.A.). Acetone and 250 mL Sigma® filter systems were purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). The acetone and ethanol were of reagent grade.

3.2.2.2 Methods

Aqueous solvent (either 70% ethanol or 70% acetone/DI water) was prewarmed at 40 °C. One-gram avocado byproduct powder (either seed or peel powder) was then added into 10 mL of the aqueous solvent. Afterwards, the powder-solvent mixture was mixed using a Vortex mixer for 30 minutes, and a Fisher Scientific® ultrasonic cleaner (Pittsburgh, PA, U.S.A., model: FS30D) for another 45 minutes. Next, an Eppendorf centrifuge (Hamburg, Germany, model: 5804 R) at 3000 rpm was used for 10 minutes at room temperature to collect supernatant (crude phenolic extract) from the powder-solvent mixture, and the supernatant was filtered using a Sigma® filter system with a 0.22 μm pore size polyethersulfone (PES) membrane. Each gram of avocado byproduct powder was extracted twice, and the supernatants were combined. Finally, solvent contained in the filtered supernatant was evaporated using an IKA® RV10 rotary evaporator (Staufen im Breisgau, Germany) at 30 °C, and the phenolic extract was stored in a dark bottle under refrigeration at 4 °C until use.

3.3 Phenolic compounds in the crude extracts of Hass avocado byproducts

3.3.1 Materials

The acetonic peel and seed extracts obtained from section 3.2.2 were evaluated for phenolic compound identification and quantification. Mass spectrometry standards used for the phenolic compound quantification analysis were purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). All the standards were of reagent grade.

3.3.2 Methods

This experiment was performed by the Mass Spectrometry Research Technology Support Facility at Michigan State University.

Phenolic compounds in both peel and seed extracts were identified and quantified using liquid chromatography/mass spectrometry (LC/MS) on a Waters Xevo® G2-XS QTof (Manchester, UK) interfaced to a Waters I-class Acquity solvent delivery system. Compounds were separated on a Waters HSS-T3 column (2.1 x 100 mm, held at 40˚C) using a gradient based on 10 mM ammonium formate adjusted to pH 2.8 using formic acid (Solvent A) and acetonitrile (Solvent B) at a total flow rate of 0.40 mL/minute. Solvent gradient was as follows (%A/%B): initial: (98/2); hold at (98/2) until 4.0 minutes, linear gradient to (90/10) at 8.0 minutes, (75/25) at 20 minutes, (5/95) at 32 minutes, with a hold until 37 minutes, followed by return to initial conditions. Mass spectra were acquired using electrospray ionization in negative-ion mode, with

a capillary voltage of -2.0 KV. Centroided mass spectra were acquired over *m/z* 100-1500 at 0.3 seconds/spectrum. Data were acquired using MS^E (alternating low- and high-energy collision conditions, collision potential was ramped from 20-80 V during high energy spectrum acquisition). Argon was used as collision gas at a manifold pressure of 1.2×10^{-1} mbar. Leucine enkephalin was used as lock mass with real-time correction of ion masses.

The Avocado byproduct extracts were diluted in Milli-Q water at the ratio of 1:10 before analysis, and injections of 10 μL were made for each sample using a Waters 2777 autosampler (Milford, MA, U.S.A.). Two cocktails of external standards were analyzed to generate calibration curves. Post-acquisition data analysis was performed using Waters QuanLynx software, integrating peak areas for $[M-H]^-$ ions. Quantitative analyses for those analytes for which authentic standards were not available, e.g. catechin isomers, were quantified using the assumption that response factors were the same as the standard with greatest structural similarity (A. Jones, personal communication, Nov. 14, 2019).

3.3.3 Phenolic compound identification and quantification results

As shown in Table 3.1 and Figure 3.1, there were 28 types of phenolic compounds identified from the avocado peel and seed extracts. The identification results, in general, agreed with the mass spectral analyses reported by Kosińska et al. (2012), Pahua-Ramos et al. (2012) and Tremocoldi et al. (2018). Based on the literature, there were only 2 out of 13 types of

phenolic compounds could be identified from both avocado peel and seed extracts, namely, catechin and epicatechin. However, the Mass Spectrometry Facility reported that 22 out of 28 types of phenolic compounds could be found in both byproduct extracts utilized for this research. The remaining 6 types of phytochemicals were presented in only one of the extracts. According to the quantification result of each phenolic compound in the mass spectra (Figure 3.1), procyanidin dimer B (I), catechin isomer 2, procyanidin trimer 2, 5-O-caffeoylquinic acid and procyanidin trimer 1 were ranked as the top five phenolic compounds in the avocado peel extract, and the avocado seed extract was rich in oxidized procyanidin trimer 2, followed by catechin isomer 2 and oxidized procyanidin trimer 1.

Table 3.1 Phenolic compounds identified and quantified from the crude extracts of Hass avocado byproducts.

Compound Name	Type of Byproduct	Content (μg/mL)	
		Peel	Seed
Catechin isomer 1	Peel & Seed	10.3	48.3
Catechin isomer 2	Peel & Seed	1101.3	273.0
Procyanidin dimer B (I)	Peel & Seed	1516.0	75.9
Procyanidin dimer B (II)	Peel & Seed	88.5	8.2
Procyanidin trimer 1	Peel & Seed	181.0	16.0
Procyanidin trimer 2	Peel & Seed	950	66.4
Procyanidin trimer 3	Peel & Seed	71.9	4.9
Alternate procyanidin dimer 1	Peel & Seed	3.7	40.8
Alternate procyanidin dimer 2	Peel & Seed	0.47	13.5
Alternate procyanidin dimer 3	Peel & Seed	25.6	33.2

Table 3.1 (cont'd)

Oxidized procyanidin trimer 1	Peel & Seed	5.9	256.0
Oxidized procyanidin trimer 2	Peel & Seed	30.6	288.0
Protocatechuic acid	Peel & Seed	1.5	4.9
3-O-caffeoyl quinic acid	Peel & Seed	0.16	5.7
4-O-caffeoyl quinic acid	Peel & Seed	4.2	139
5-O-caffeoyl quinic acid	Peel & Seed	425.8	7.0
5-O-p-coumaroyl quinic acid	Peel & Seed	0.46	8.1
Quercetin 3-O-galactoside	Peel & Seed	3.5	0.32
Quercetin 3-O-glucoside	Peel & Seed	1.3	0.38
Quercetin-3-O-rutinoside (rutin)	Peel & Seed	16.9	0.19
Quercetin rhamnoside hexoside 2	Peel & Seed	1.0	0.36

Table 3.1 (cont'd)

Quinic acid	Peel & Seed	130.0	79.7
Kaempferol	Peel	0.01	N/A
Kaempferol dihexoside	Peel	3.7	N/A
Caffeoyl quinic acid isomer 4	Seed	N/A	25.1
Catechin isomer 3	Seed	N/A	37.0
3-O-p-coumaroyl quinic acid	Seed	N/A	13.3
4-O-p-coumaroyl quinic acid	Seed	N/A	31.3

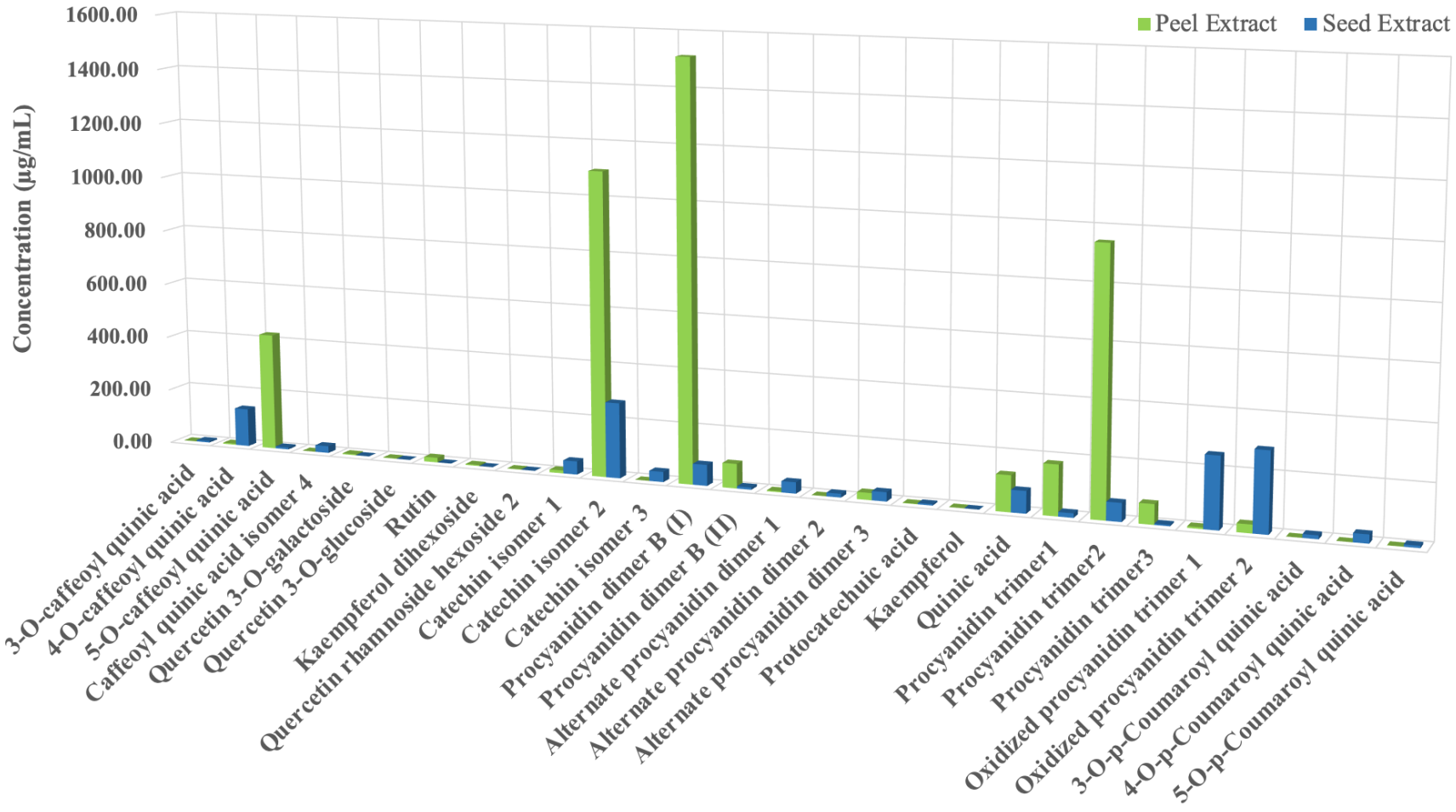


Figure 3.1 Phenolic compounds identification and quantification results for the crude extracts of Hass avocado byproducts.

CHAPTER FOUR:

Non-metal Contact Coating Process

4.1 Introduction

Phenolic compounds can be coated onto material surfaces via an oxidative polymerization reaction (Roman, Decker, and Goddard 2016). Due to the significant similarities of the chemical structures between phenolic compounds and mussel foot proteins (Mfps), phenolic compounds, after polymerization, present adhesive properties (Sileika et al. 2013).

Mfps are widely known because of their durable and strong adhesive properties (Kord Forooshani and Lee 2017). These bio-based proteins enable marine mussels to stick to foreign surfaces in wet, dry and salty environments (Lee, Lee, and Messersmith 2007; Waite 1987; Zhao et al. 2006). It is believed that 3,4-Dihydroxyphenylalanine (DOPA), as an amino acid in Mfps, plays a vital role in the adhesive actions. A DOPA molecule contains a catechol side chain (see Figure 4.1). This adhesive moiety can be involved in a) redox activities, b) metal chelating reactions, c) cross-linking actions, and d) interfacial activities (covalent and noncovalent) to allow Mfps to tightly bind to all types of materials under water, such as glass, plastics, and metal oxides (d'Ischia and Ruíz-Molina 2017; Lu et al. 2013; Mian and Khan 2017). After the adhesion on foreign surfaces, marine mussels are almost motionless. It is hard to remove them.

According to the literature, the tenacity (a size independent detachment force) of a California mussel (Mytilus Californianus) in the perpendicular direction was up to 300 N and in the parallel direction was 180 N (Mian and Khan 2017).

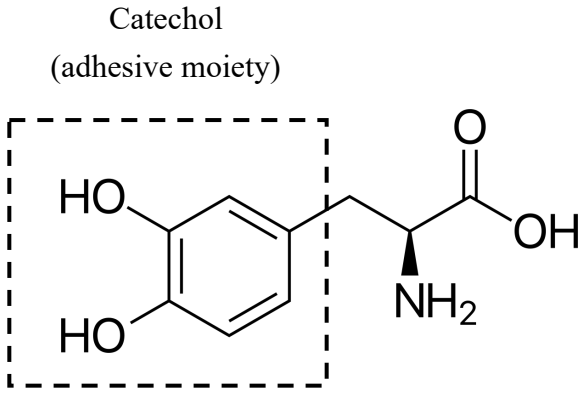
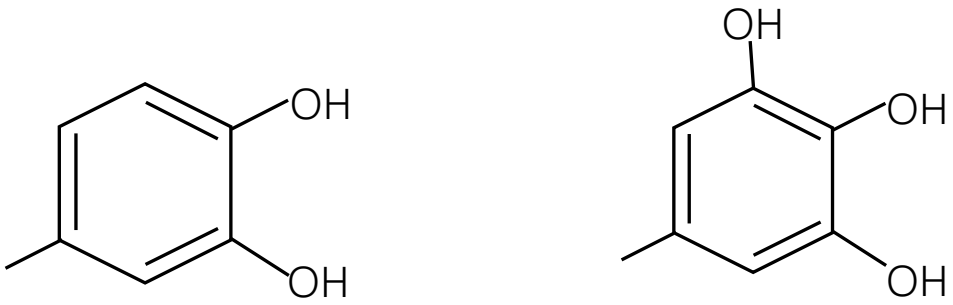


Figure 4.1 Chemical structure of a DOPA molecule.

Phenolic compounds contain catechol and/or gallol (1,2,3-trihydroxyphenyl, see Figure 4.2 and Figure 2.1 for examples on phenolic compounds) functional parts. Similar to DOPA, these side chains in the antioxidant molecules have interfacial binding properties and thus serve as strong and versatile adhesive moieties attaching phenolic compounds to various foreign surfaces. The foreign surfaces can be either organic or inorganic, such as metals, ceramics, plastics, glass, and biological materials (Barrett, Sileika, and Messersmith 2014; Forooshani, Meng, and Lee 2017; Sileika et al. 2013; Zhan et al. 2017).

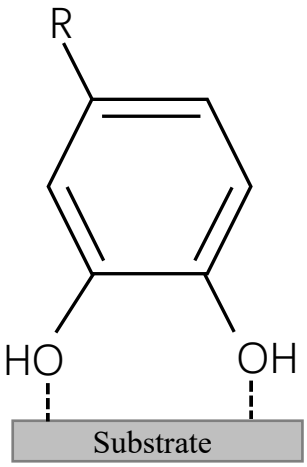


Catechol side chain Gallol side chain

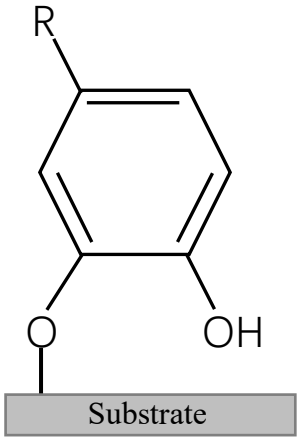
Figure 4.2 Chemical structures of a catechol and a gallol side chain.

Catechol has strong adaptability to foreign surfaces. Based on the catechol content in phenolic compounds, the nature of substrates, and the pH value of surrounding environment, catechol-contained compounds interact with foreign surfaces in four ways, i.e., a) hydrogen bonding, b) coordination (monodentate, bidentate, and chelating bidentate), c) π - surface interaction (π - π and π - cation interaction) and d) covalent bonding via Michael-type addition (see Figure 4.3, Andersen, Chen, and Birkedal 2019; Saiz-Poseu et al. 2019).

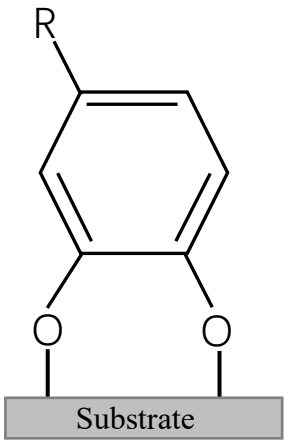
The same interfacial activities were reported between gallol-containing compounds and foreign surfaces. In addition to bidentate coordination, tridentate coordination was noticed. Compared with bidentate coordination, tridentate interfacial activity resulted in stronger binding strength (Zhan et al. 2017).



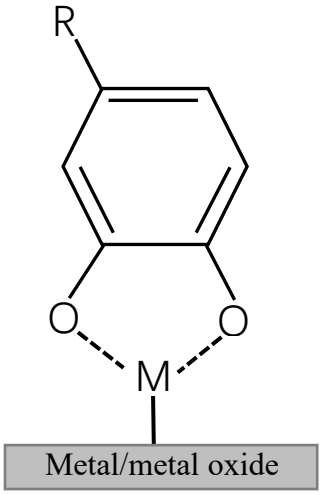
a) Hydrogen bonding



b) Monodentate



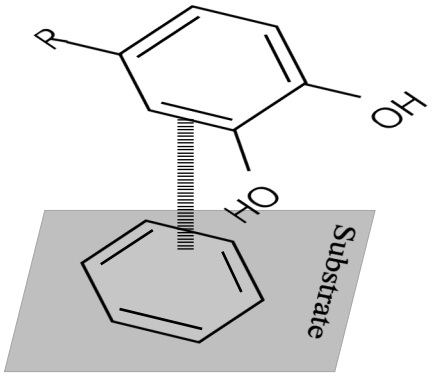
Bidentate



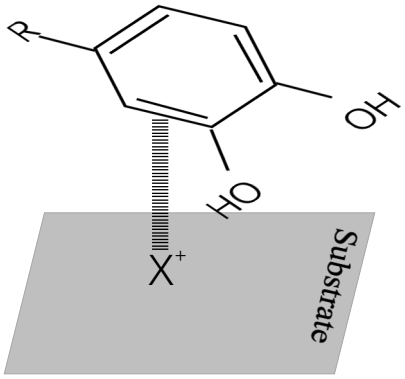
Chelating bidentate

Figure 4.3 Schematic of interfacial activities between catechol-contained compounds and foreign substrates. R represents the remainder of a catechol-containing molecule, M represents a metal atom in a substrate, X^+ represents a cation in a substrate.

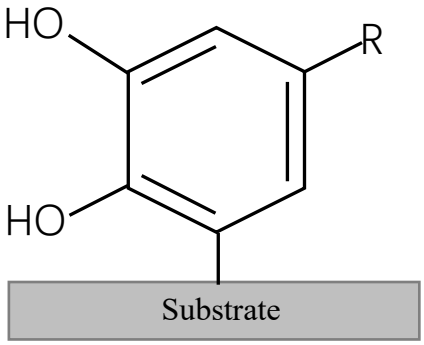
Figure 4.3 (cont'd)



c) π - π interaction



π - cation interaction



d) Covalent bonding (via Michael-type addition)

There are two mechanisms suggested in the literature as coating methods for phenolic compounds: laccase assisted enzymatic polymerization (Jeon et al. 2010, 2013) and alkaline saline assisted oxidative polymerization (Geißler et al. 2016; Sileika et al. 2013). For both methods, oxygen involvement and moderate mechanical agitation are required. These two approaches utilize coating solutions with different pH values and different catalysts to

polymerize phenolic compounds onto flexible films. For the laccase assisted coating method, researchers proposed adding laccase obtained from *Trametes versicolor* into an acid buffer solution (pH = 5) to expedite the phenolic compound polymerization process, whereas the alkaline saline assisted method used an alkaline buffer solution (pH = 7.8) to complete the phenolic compound polymerization process. In the presence of sodium chloride, the coating efficiency of the alkaline saline method could be enhanced. Considering the requirement of enzymes for the laccase assisted approach, the coating cost is much higher than that of the alkaline saline method.

Roller coating is a conventional process to apply a coating layer onto a flexible plastic substrate. Basically, it utilizes a metal roll to fully contact with the surface of a substrate, and thus physically transfer a coating layer from the roll to the substrate. The thickness of the coating layer and the coating speed are adjustable. Due to the high transfer efficiency and minimal labor requirement, roller coating is widely accepted. It is an ideal method for stable coating solutions that do not react with metals.

In addition to free radical scavenging and electron-donating abilities, polymerized phenolic compounds (polyphenols) are metal chelators (Chew et al. 2008). They can be utilized to chelate ferrous, ferric, and cupric ions in different environments with various pH values. The binding abilities of the phytochemicals to different metals depend on their phenolic structure and

the location of their hydroxyl groups (Senevirathne et al. 2006; Thompson, Williams, and Elliot 1976).

To avoid the metal chelating reaction happening during the coating process, conventional roller coating process was not considered as a feasible method for this research. A non-metal contact coating system with an agitation feature was developed.

4.2 Materials

4.2.1 Chemicals

Sodium chloride, sodium hydroxide, bicine, and glacial acetic acid were purchased from VWR International (Radnor, PA, U.S.A.). Sodium acetate and laccase from *Trametes versicolor* were purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). All the chemicals were of reagent grade.

4.2.2 Sample films

LDPE film was selected from the polymer films available in the School of Packaging (East Lansing, MI); the manufacturer was unknown. Biaxially oriented PP and oriented PET film were supplied by Dow Chemical Company (Midland, MI, U.S.A.).

By using Dyne test pens, the surface energy of the PP and PET films were determined to be 30 and 34 dyne/cm, and the surface energy of the LDPE film was lower than 30 dyne/cm. The thickness of the LDPE, PP and PET films were 38.80 ± 0.45 μm, 20.00 ± 1.00 μm, and 14.20 ±

1.30 μm respectively, determined by averaging five measurements using a TMI digital micrometer (Ronkonkoma, NY, U.S.A., model number: 49-70-01-001).

4.2.3 Coating device

Figures 4.4 and 4.5 present the schematics of the non-metal contact coating device developed for this research. It is a dip coating system. Basically, it utilizes a slider-crank mechanism to convert rotary motion to linear motion.

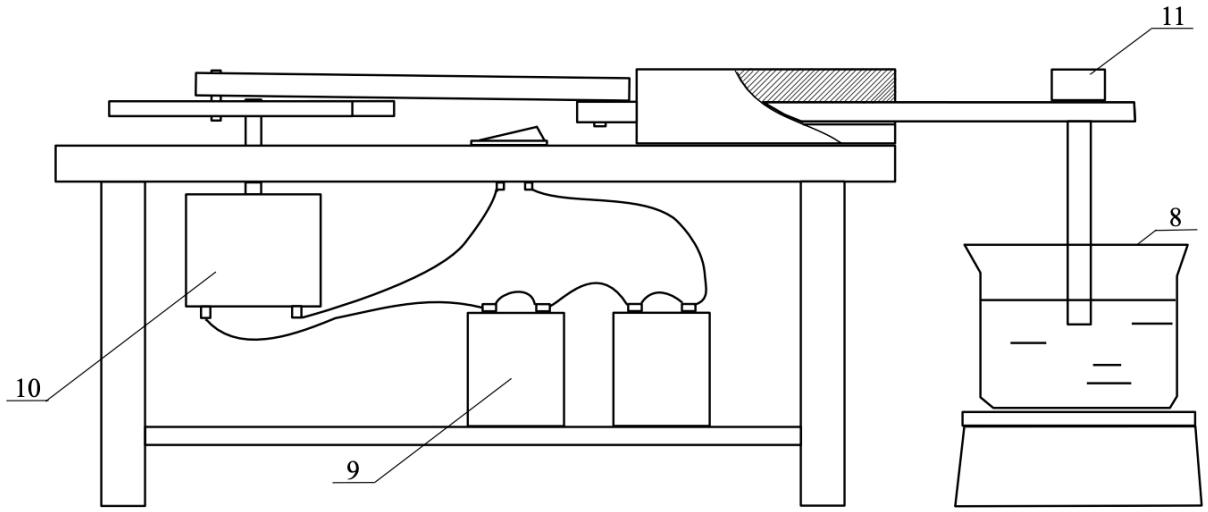
There are two ways of linear motion designed for the coating process, i.e., horizontal and vertical movements. A sample film can be either horizontally moved inside the coating solution by using the connecting bar attached to the rotation bar and slider (Figure 4.4), or, it can be vertically moved if the connecting bar is removed (Figure 4.5). Both linear movements allow the involvements of oxygen from the atmosphere and moderate agitation during the coating process to meet the polymerization requirements. The movement's frequency is determined by the speed of the electric motor underneath the rotation bar. In other words, the dip coating speed is a controllable variable.

The diameters of different beakers used for coating are not the same and limit the moving range of a sample film inside the coating solution if horizontal movement is required. The connecting bar is designed to attach to different fitting holes on the rotation bar based on beaker

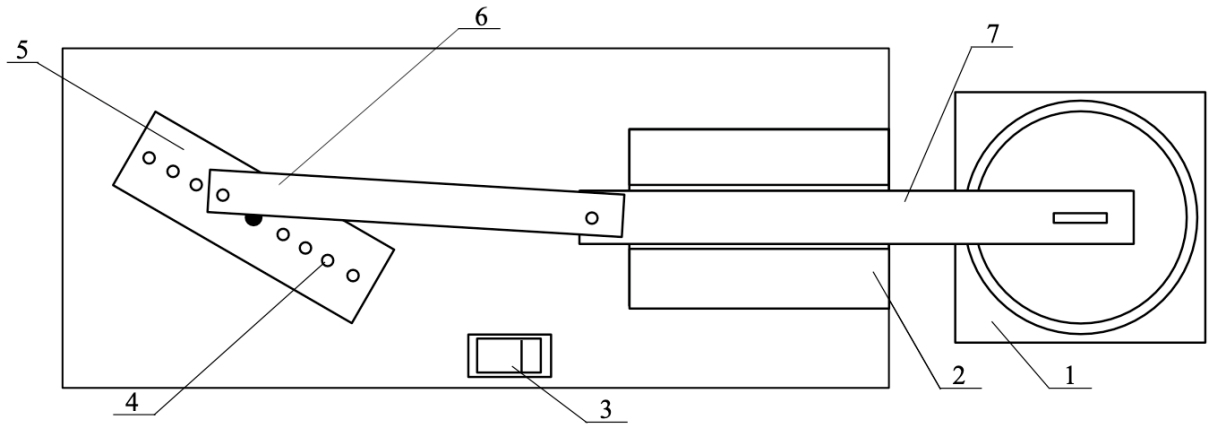
size to make sure the sample film movement is within the range, allowing different sizes of vessels to be used.

For this research, the vertical movement design was employed based on the preliminary results of coating tests.

To meet the agitation requirement for the coating process, in addition to using the sample holder to stir the coating solution while dip coating a sample film, the hot plate stirrer placed under the beaker can also agitate the solution while controlling the coating temperature.



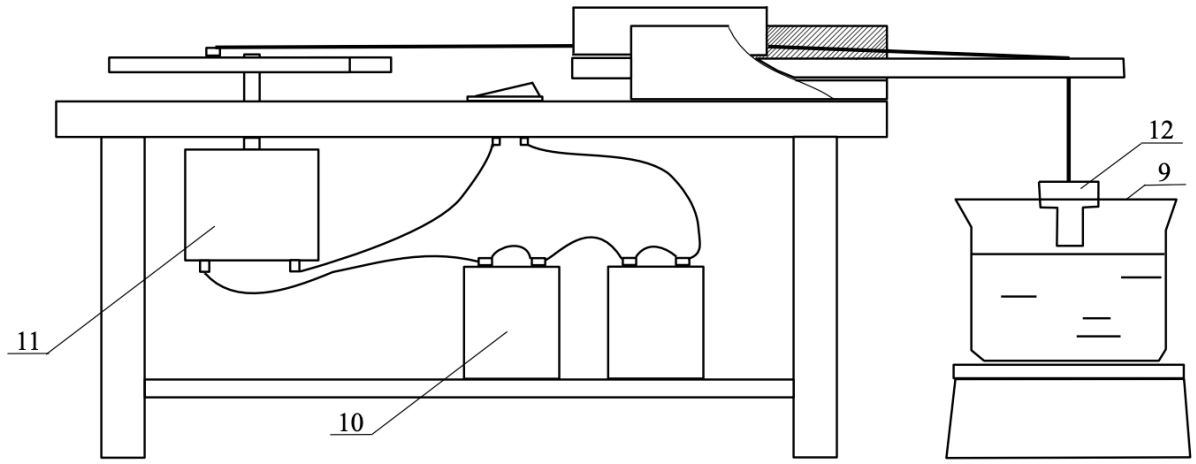
A. Front View



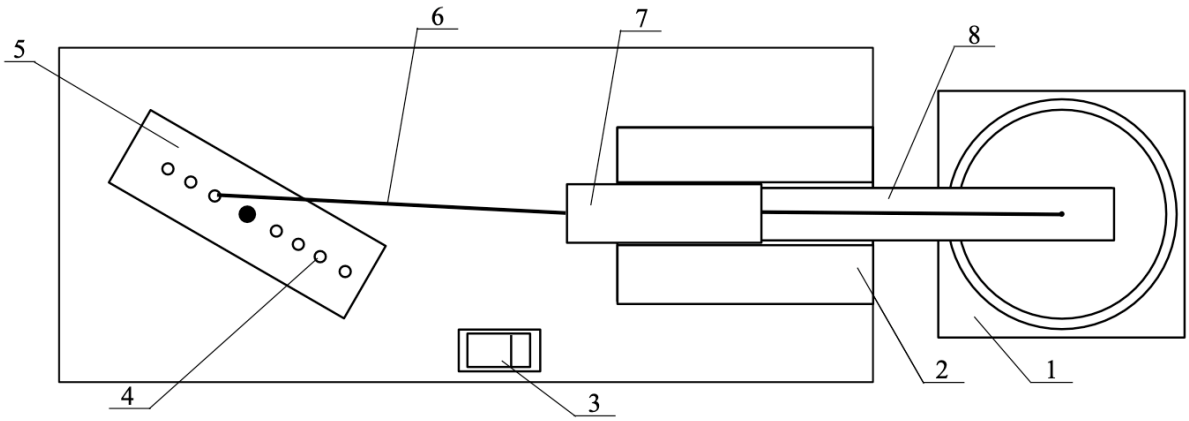
B. Top View

1	Hot plate stirrer
2	Guides A
3	Power switch
4	Fitting holes
5	Rotation bar
6	Connecting bar
7	Slider
8	Beaker
9	Electrical energy
10	Electric motor
11	Sample holder

Figure 4.4 Schematic of the non-metal contact coating device with horizontal movement.



A. Front View



B. Top View

1	Hot plate stirrer
2	Guides A
3	Power switch
4	Fitting holes
5	Rotation bar
6	Slider
7	Guide B
8	Fixed guide
9	Beaker
10	Electrical energy
11	Electric motor
12	Sample holder

Figure 4.5 Schematic of the non-metal contact coating device with vertical movement.

4.3 Methods

4.3.1 Laccase assist coating (sodium acetate)

Glacial acetic acid was used to adjust the pH value of 0.1 M sodium acetate buffer to 5. Then, the crude phenolic extract (100% peel extract, 100% seed extract, or 50% peel extract and 50% seed extract) was dissolved into the buffer solution at a concentration of 25 mg/mL, followed by adding 1 mg/mL laccase. Afterwards, a 4.5 × 2 cm sample film was rinsed with DI water, purged by pure nitrogen flow, and attached to the sample holder of the coating device for 24-hour dip coating at 23 °C. To meet the mild agitation requirement of the coating process, the dipping speed of the coating device (the speed of the electronic motor) was 25 rpm, and the coating solution stirring speed of the hot plate (Scilogex LLC, Rocky Hill, CT, U.S.A., model: MS-H280-Pro) was set at 350 rpm. Finally, the coated sample film was detached from the sample holder, rinsed with DI water, and purged by pure nitrogen flow again to remove any residue on the film surface.

4.3.2 Alkaline saline coating (bicine)

This coating method was based on the experimental design proposed by Geißler et al. 2016. In essence, the coating buffer was made by mixing 0.1 M bicine (end concentration) with 0.6 M NaCl (end concentration). The pH value of the buffer solution was then adjusted to 7.8 using NaOH. To make the final coating solution, the crude phenolic extract (100% peel extract,

100% seed extract, or 50% peel extract and 50% seed extract) was added into the buffer solution at a concentration of 25 mg/mL. Afterwards, a 4.5 × 2 cm sample film was rinsed with DI water, purged by pure nitrogen flow, and attached to the sample holder of the coating device for 24-hour dip coating at 23 °C. To meet the mild agitation requirement of the coating process, the dipping speed of the coating device (the speed of the electronic motor) was 25 rpm, and the coating solution stirring speed of the hot plate was set at 350 rpm. Finally, the coated sample film was detached from the sample holder, rinsed with DI water, and purged by pure nitrogen flow again to remove any residue on the film surface.

4.3.3 Sample preparation for scanning electron microscope (SEM) observation

To understand the distribution of the antioxidant coating on the substrates, coated LDPE, PP and PET films were used for surface and cross-sectional SEM observations, and a coated glass coverslip (diameter: 12 mm, # 1) was also used for coating thickness measurement. All the substrates were coated with the acetonic avocado peel extract by following the alkaline saline coating method stated in section 4.3.2. The reason for choosing this coating method will be discussed in chapter 5.

4.3.3.1 Sample preparation for surface observation

At room temperature, a coated polymer film was cut into 1 × 1 cm samples with a utility knife, and then attached to an aluminum SEM specimen stub (diameter: 25 mm) using clear epoxy adhesive (see Figure 4.6).

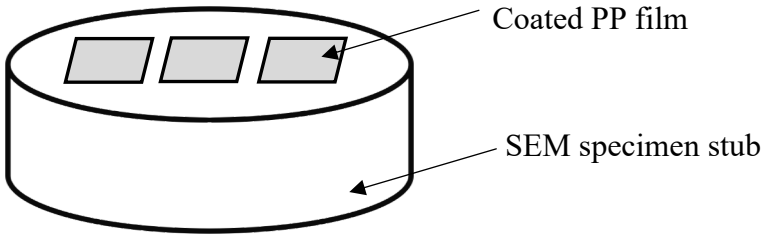


Figure 4.6 SEM sample preparation for surface observation of a coated polymer film.

4.3.3.2 Sample preparation for cross-sectional observation and thickness measurement

To minimize deformation of the antioxidant coating caused by the sample preparation process, liquid nitrogen was used to temporarily freeze the coated substrates and a SEM specimen stub.

As shown in Figure 4.7, a 25 mm stub was first placed into liquid nitrogen for 2 ~ 3 minutes; then, a coated polymer film was placed into liquid nitrogen for around 30 seconds. After that, the SEM stub and coated PP film were removed from the liquid nitrogen. A new razor blade was used to immediately cut the temporarily frozen film into small samples on the

temporarily frozen SEM stub. Finally, the sample films were vertically attached onto a 12.5 mm SEM specimen stub by using clear epoxy adhesive. For a clear cross-sectional view under SEM, a new razor blade was used for each cut.

The coated glass coverslip was treated in the same way. Rather than using a razor blade, the frozen coverslip was manually fractured into small pieces after removing it from the liquid nitrogen.

After curing for 15 hours the epoxy adhesive, all the SEM samples were added a thin layer of iridium as the conductive coating.

The SEM photos for both surface and cross-sectional observations were obtained by placing the coated films and glass coverslips into the chamber of a JEOL JSM 7500F (JEOL Ltd., Tokyo, Japan), and visualized under an accelerating voltage of 5 kV.

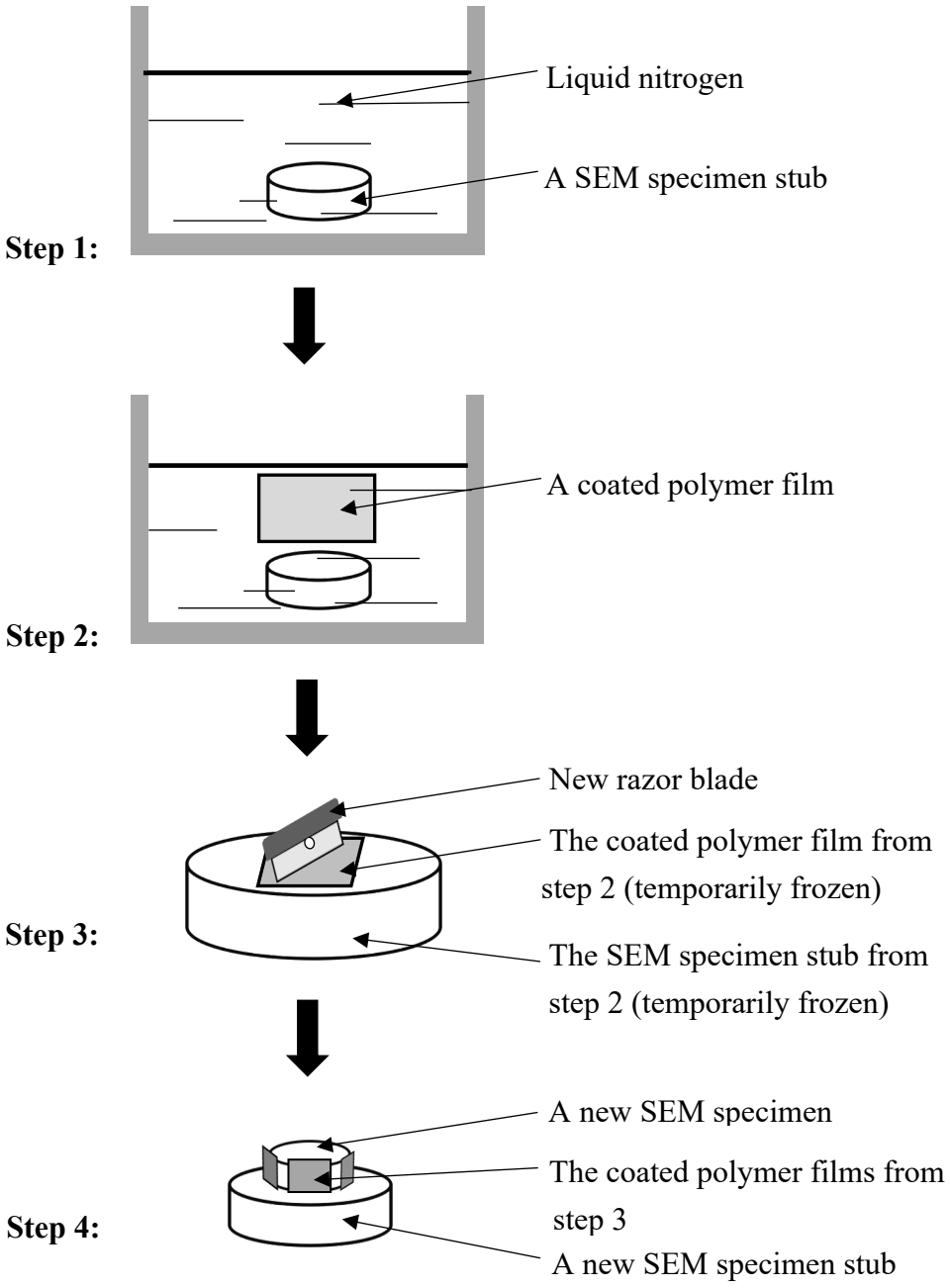


Figure 4.7 SEM sample preparation for cross-sectional observation of a coated polymer film.

4.4 Antioxidant coating layer under SEM

Figures 4.8, 4.9 and 4.10 illustrate the surface SEM images of the antioxidant coating on a PP, LDPE and PET film, respectively. The cracked texture was caused by the conductive coating layer, iridium, at a high level of resolution. As reflected in the images, each coating surface presented in almost the same grayscale. In addition, no charge-contrast spots, air bubbles, gaps, pinholes and fisheyes were noticed. These phenomena implied that the phenolic compounds from the avocado peel extract did not form small clumps on the film surface during the alkaline saline coating process; instead, they were uniformly distributed. This finding was later supported by the cross-sectional images of the coated polymer films (Figures 4.11, 4.12 and 4.13). While observing the surface characteristic of the coated LDPE film, the coating layer actively interacted with the electron beam of SEM, resulting in wrinkles on the film surface within 5 seconds. A better preparation method should be developed for surface observation of the coated LDPE film to avoid sample deformation.

While observing the antioxidant coating from its cross-sectional view, the SEM images did not exhibit a sharp and clear distinction between the substrates and the coating layer. Possibly, the razor blade, when cutting the coated films during the SEM sample preparation process, compressed the coating layer and thus caused deformation of the coating on its cross-sectional surface. Based on Figures 4.11, 4.12 and 4.13, the deformation caused the edge of the phenolic coating to stretch on its substrate. In this circumstance, measuring the coating thickness

was challenging. Therefore, a glass coverslip was used to replace the polymer films as the coating substrate. Under SEM, a clear edge line was found between the coating layer and the glass coverslip (Figure 4.14). The phenolic compounds were evenly polymerized on the glass coverslip, which indicates a great potential of using other types of materials as the substrate coated with the avocado peel extract by employing alkaline saline coating method. By averaging the coating thickness at 4 different locations, the coating thickness was determined to be 37.75 ± 0.30 nm.

It was noticed that the SEM sample preparation method for the coated glass coverslip resulted in deformation at some spots of the coating layer. At high magnification (35000x), clumps were observed at some spots of the coating layer surface. This deformation might be introduced by the SEM sample preparation process. After removal from liquid nitrogen, the coated glass coverslip became very brittle. Manually fracturing the sample, in this case, might result in deformation at some spots of the coating layer. Therefore, a better preparation method should be developed for thickness measurement of future samples to avoid sample deformation.

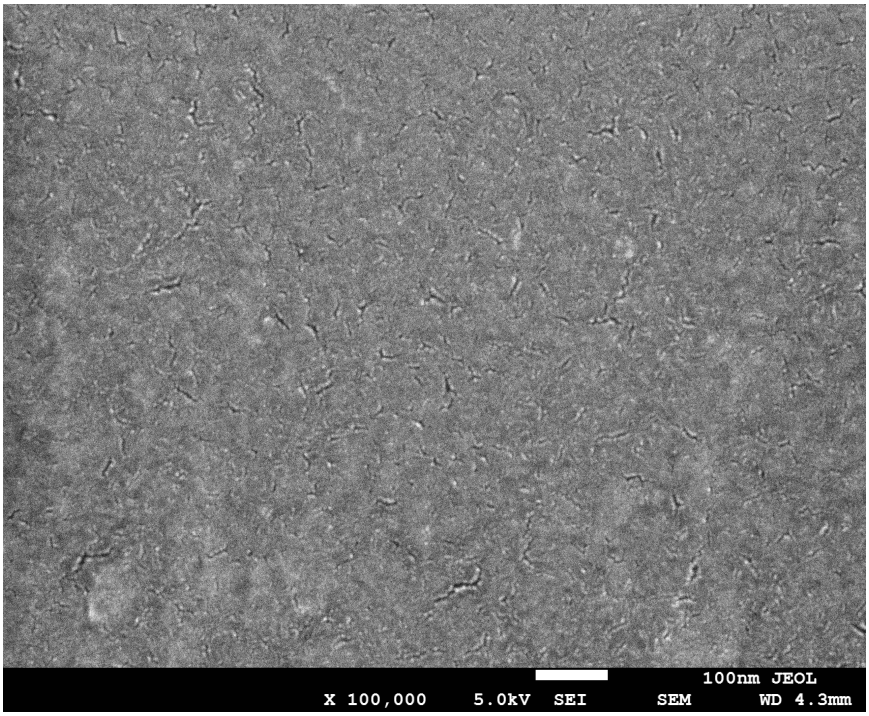


Figure 4.8 Topside view of the phenolic coating on a PP film.

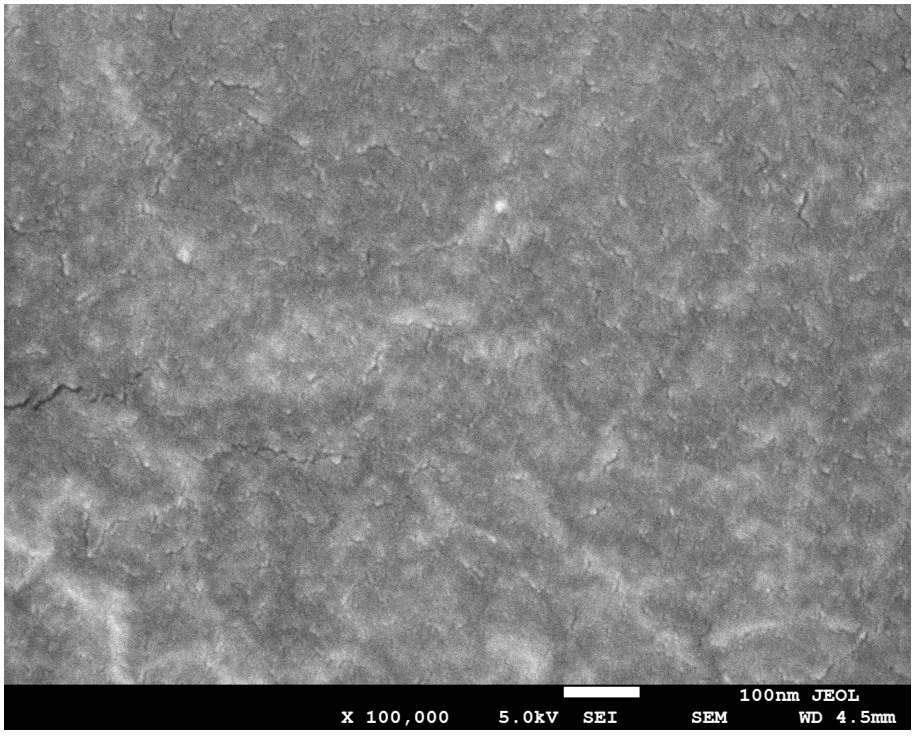


Figure 4.9 Topside view of the phenolic coating on a LDPE film.

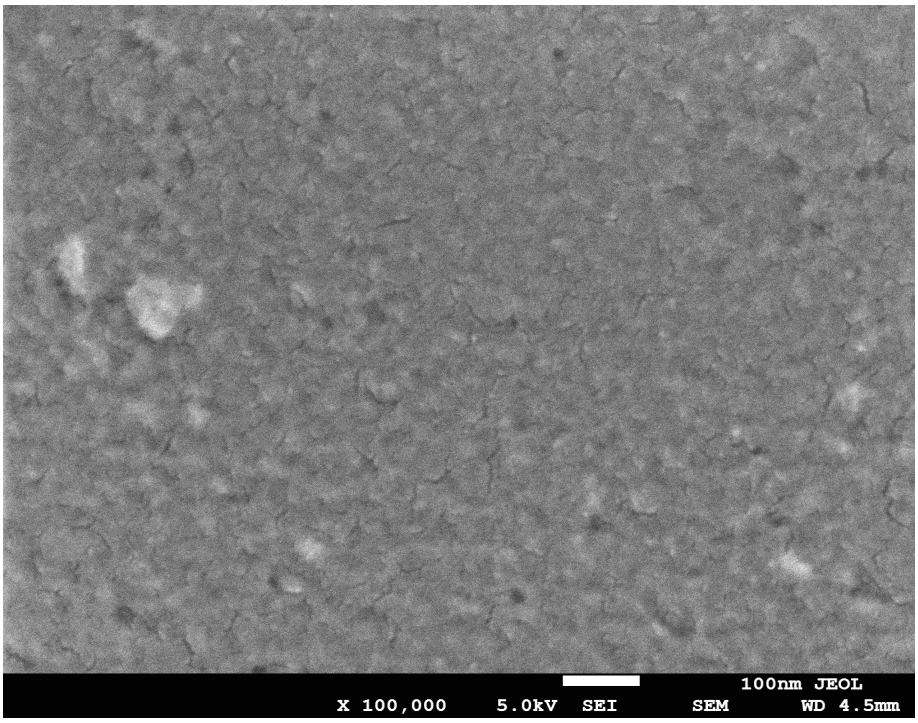


Figure 4.10 Topside view of the phenolic coating on a PET film.

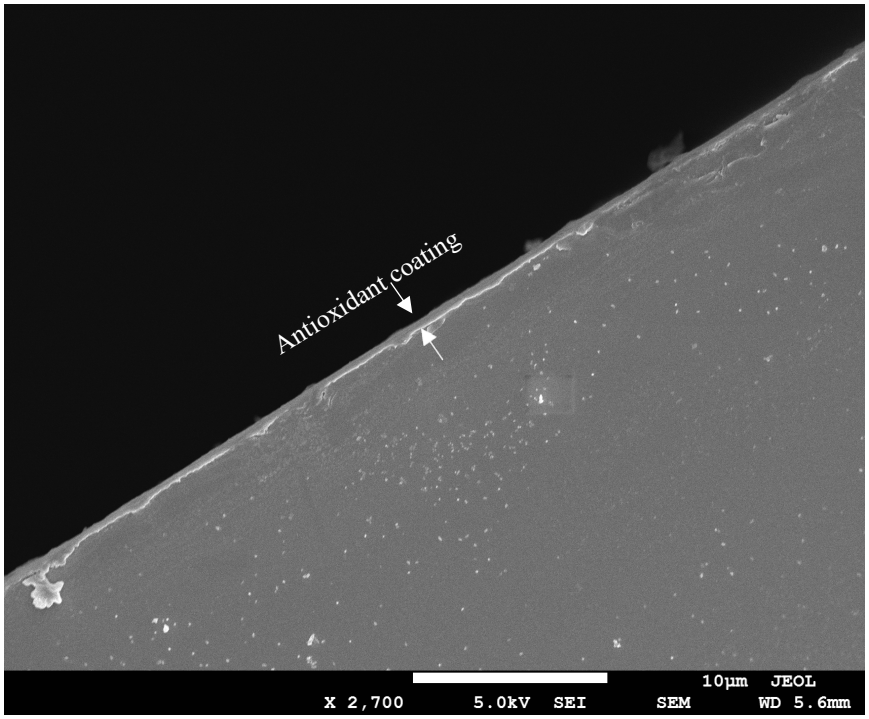


Figure 4.11 Cross-sectional view of the phenolic coating on a PP film.

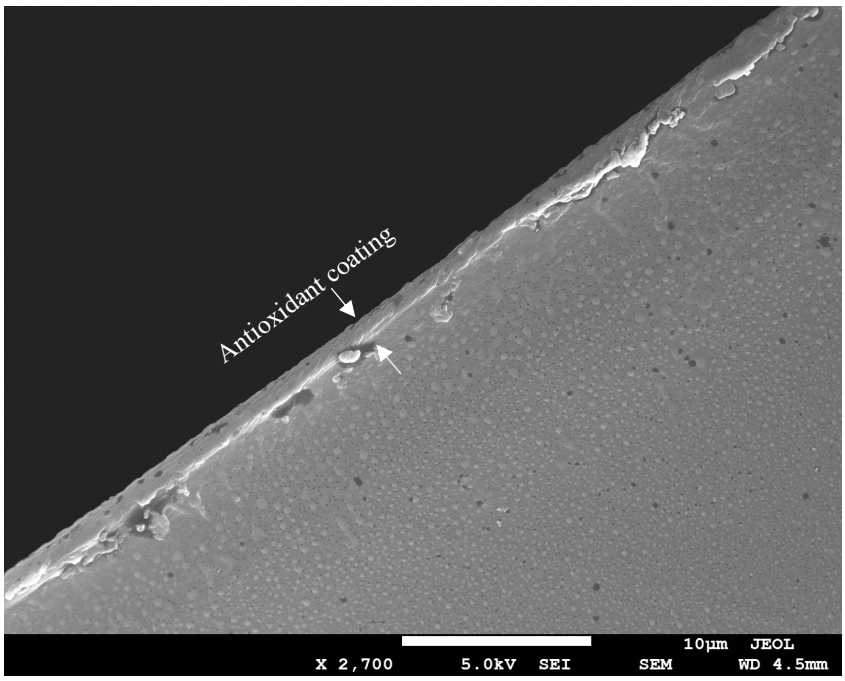


Figure 4.12 Cross-sectional view of the phenolic coating on a LDPE film.

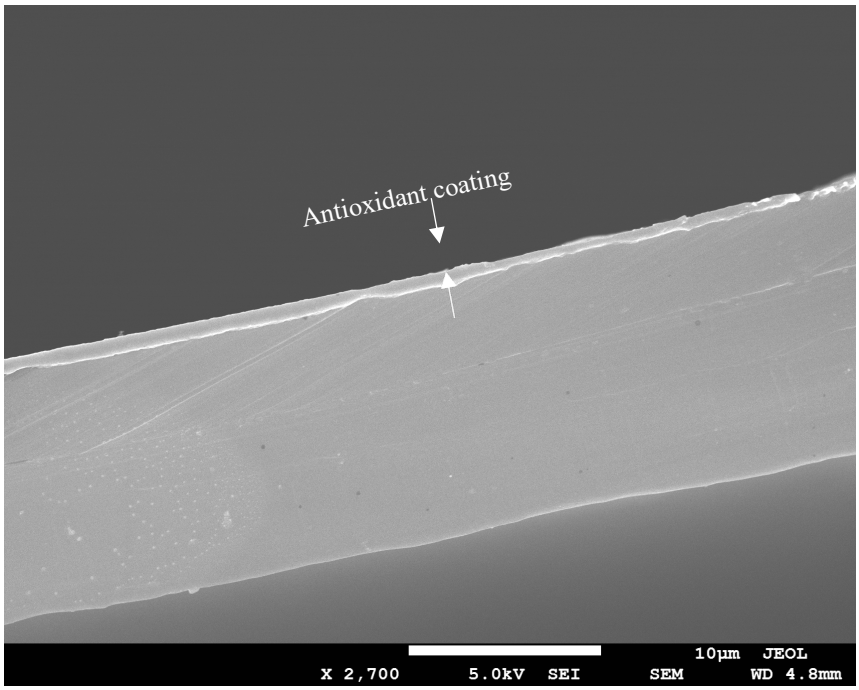


Figure 4.13 Cross-sectional view of the phenolic coating on a PET film.

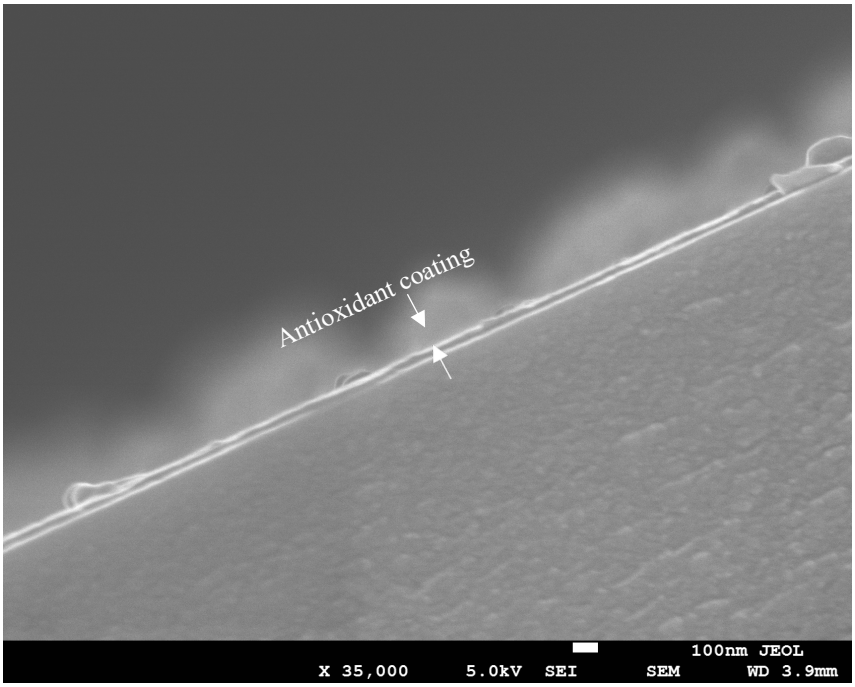


Figure 4.14 Cross-sectional view of the phenolic coating on a glass coverslip.

To uniformly attach phenolic compounds from the avocado byproduct extracts onto nonpolar LDPE and PP films, and PET film with low surface energy (34 dyne/cm), two types of interfacial reactions might be initiated during the coating process, namely, covalent bonding via Michael-type addition and π - surface interaction.

For the LDPE and PP films, covalent linkage (Michael-type addition) could occur between catechol functional group(s) of the phenolic compounds and the film surfaces. In the presence of oxygen, the adhesive moiety of phenolic compounds could be firstly oxidized to quinone form. After that, carbons in the polymeric chains of LDPE or PP might serve as nucleophiles to react with the oxidized adhesive moiety in the wet environment. In this way, the

phenolic compounds could finally attach to the film surfaces, see Figure 4.15. Similar mechanisms were reported by Saiz-Poseu et al. (2019) and Yang, Stuart, and Kamperman (2014).

For the PET film, the interfacial reaction might not be limited to covalent linkage only. Considering the existence of aromatic rings in the polymeric chain of PET, π - surface interaction (Figure 4.16) might also be initiated during the coating process to allow the phenolic compounds to anchor on the film surface.

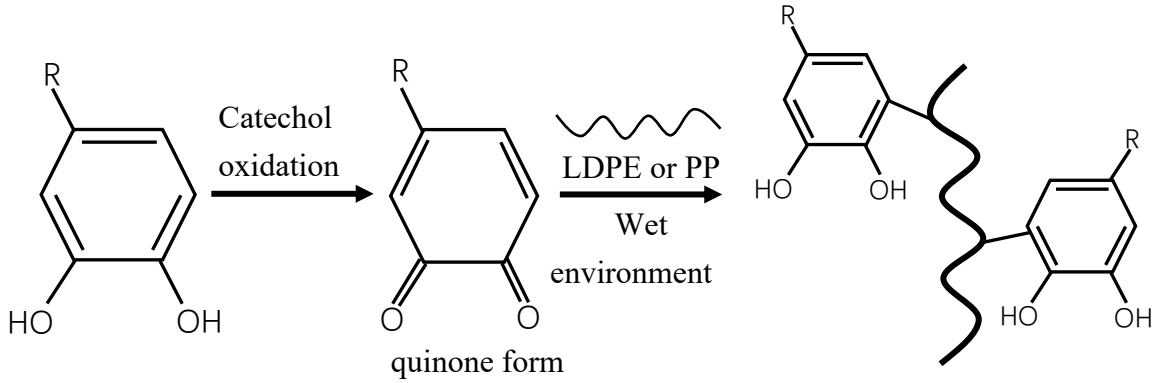


Figure 4.15 Schematic of covalent linkage between catechol-contained phenolic compounds and a LDPE or PP film surface. R represents the remainder of a catechol-containing phenolic compound.

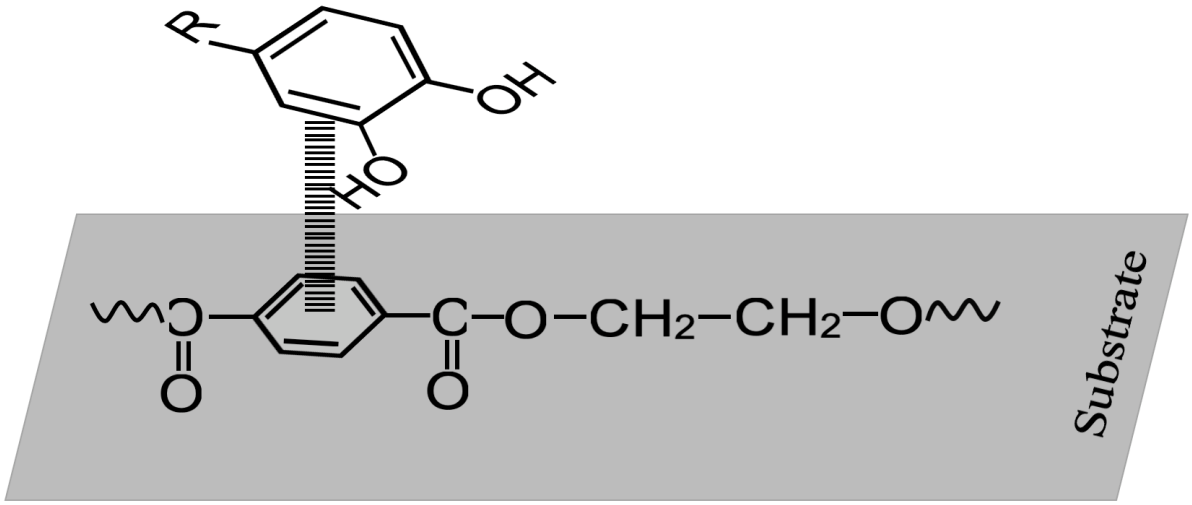


Figure 4.16 Schematic of π - surface interaction between a catechol-contained phenolic compound and a PET film surface.

CHAPTER FIVE:

Evaluation of Antioxidant Activity of Coated Polymer Films

Part I: Screening Tests

5.1 Introduction

Before determining the potential applications of plastic substrates coated with the phenolic compounds extracted from avocado byproducts, three types of screening tests were employed to evaluate the antioxidant efficacy of the phenolic coating. By applying the laccase assist coating (section 4.3.1) and alkaline saline assist coating (section 4.3.2) methods, the phenolic content from avocado byproducts was attached onto PP, PET and LDPE films for inspection. Both the ethanolic and acetonic extracts from avocado peel and seed (section 3.2.2) were utilized.

At the first stage, silver nitrate ($AgNO_3$) solution was used for visually inspecting the existence of the phenolic coating. In contact with this solution, the phenolic coating acted as a reducing agent to convert Ag^+ to Ag^0, and excited surface plasmon resonance. The excitation, in this way, changed the coating to a dark yellow or brown color indicating the presence of phenolic content.

At the second stage, a DPPH• assay was used. The phenolic coating served as an electron donor to stabilize the free radicals reducing the concentration of DPPH• in the working solution.

The potential influences of the coating environment, the concentration of phenolic extract in the coating solution, and different plastic substrates on the antioxidant effectiveness of the coating layer were investigated.

An ABTS•+ assay, as the third evaluation method, was employed to confirm the experimental results obtained from the previous stages. In this reaction, the phenolic coating transferred hydrogen atoms to ABTS•+ to decrease the radicals in the working solution. The potential influences of the coating environment and different plastic substrates on the antioxidant efficacy of the coating layer were studied. In addition, the avocado peel and seed extracts were directly evaluated to understand their antioxidant potency to quench ABTS•+ radicals in the working solution.

For both the DPPH• and ABTS•+ assays, food simulants (95%, 50%, and 10% aqueous ethanol) were used to analyze the antioxidant performance of the phenolic coating from avocado byproducts.

5.1.1 $AgNO_3$

5.1.1.1 Introduction

In the nanotechnology field, food and food byproduct extracts are utilized as reliable sources to synthesize silver nanoparticles (AgNPs). Compared with the chemical and physical synthesis methods, this biological technique provides an environment-friendly alternative to

easily obtain silver nanoparticles in a more economical way (Ibrahim 2015; Mohanpuria, Rana, and Yadav 2008).

Silver nanoparticles are non-toxic and inorganic. They can serve as great antibacterial agents to kill around 650 disease-causing organisms in the body (S. H. Jeong, Yeo, and Yi 2005), as excellent sterilizers and UV-protectors on cotton fabric (Rai et al. 2014), and as a spectrally selective coating for solar energy absorption (Mohanpuria et al. 2008). The potential applications of this nanoparticle have been extensively studied in recent years.

To synthesize AgNPs, food and food byproduct extracts need to react with aqueous silver nitrate solution. During this reaction, the extracts are used as reducing agents to convert Ag^{+} to Ag^{0}, and thus cause metal deposition (Jeeva, Thiyagarajan, Elangovan, Geetha, & Venkatachalam, 2014). Other than metal deposition, the reduction process activates the excitation of surface plasmon resonance, resulting in color change of the solution (Mulvaney 1996; Sosa, Noguez, and Barrera 2003). Normally, a dark yellow or brown color can be observed indicating the existence of biosynthesized silver nanoparticles.

There is no universal condition that works for all the biosynthesis reactions of silver nanoparticles. The silver ion reduction potential is mainly determined by reaction time, solution temperature, the concentrations of aqueous silver nitrate solution, and the volume of natural extracts. According to the literature, the reaction time used for the synthesis of AgNPs varied

from 5 minutes to 72 hours; the solution temperature varied from room temperature to 100 °C; the range of aqueous silver nitrate concentration was from 0.25 mM to 5.0 mM, and from 0.25 mL to 3 mL of natural extracts were used to reduce silver ions (Ibrahim 2015; Jeeva et al. 2014; Jeon et al. 2013; Sileika et al. 2013).

In the natural extracts employed for the synthesis of silver nanoparticles, phenolic compounds were evaluated as effective ingredients. By utilizing phenolic compounds extracted from rice husk, *Satureja intermedia* C.A. Mey, and Ananas comosus, Liu et al. (2018), Firoozi et al. (2016), and Ahmad et al. (2012) reported the formation of AgNPs from the silver ion reduction test. This indicated the possibility of utilizing aqueous silver nitrate solution to inspect for the presence of phenolic compounds.

Hence, aqueous silver nitrate solution was employed, for this research, to visually inspect for the presence of the phenolic compounds, which were extracted from avocado byproducts, in the coating layer of the sample films (based on color change of the coated films). Trial-and-error was used to determine the reaction time, solution temperature and concentration of the aqueous silver nitrate solution.

5.1.1.2 Materials

Silver nitrate powder was purchased from VWR International (Radnor, PA, U.S.A.). This chemical was of reagent grade.

Ethanolic seed extract was utilized to coat PP, PET, and LDPE films by applying the laccase assist coating (section 4.3.1) and alkaline saline assist coating (section 4.3.2) methods.

5.1.1.3 Methods

PP, PET and LDPE films coated with the phenolic layer were immersed into aqueous silver nitrate solution (100 mM) for at least 24 hours at room temperature. After that, the sample films were removed from the silver nitrate solution, rinsed with DI water, and purged by pure nitrogen flow. For comparison purposes, uncoated PP, PET and LDPE films were used as control samples and treated in the exact same way. Both the coated and uncoated films were photographed for evaluation.

5.1.1.4 Results and discussion

After immersing the coated polymer films into silver nitrate solution, a slight color change was observed on the sample films after 24 ~ 48 hours. Starting from a light-yellow color, the coated sample films gradually changed to a brown or greyish brown color as time increased. This implied the deposition of silver nanoparticles on the surfaces of sample films, and the excitation of surface plasmon resonance resulted from the silver ion reduction process.

For each coated sample film, the color evolution process did not start at the same time. Generally, the polymer films coated in the alkaline saline solution initiated the color change first. After 24-hour immersion, a noticeable light-yellow color was perceived on the surface of sample

films coated in the alkaline solution. On the other hand, it took at least 48 hours for the sample films coated in the laccase assist solution to start changing their color. At the end of the reaction, a strong color contrast was observed between the films coated in the alkaline solution and the laccase assist solution (Figure 5.1). Dark yellowish or greyish brown colors showed on alkaline solution coated sample films, while a light brown color presented on laccase solution coated sample films. This might imply that the alkaline saline solution, which is a comparatively low cost coating solution, could provide more potent phenolic coating on the sample films. Other evaluation methods were required to further support this statement.

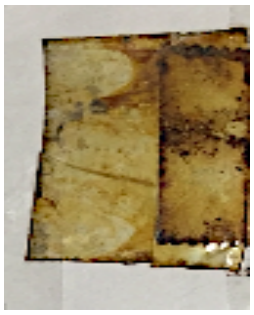
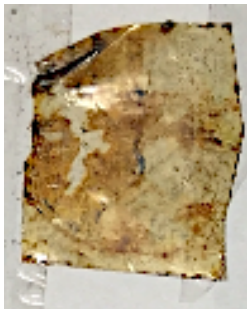
Film / Experimental Condition	PP	PET	LDPE
Control group			
Coated in laccase assist solution (pH = 5)			
Coated in alkaline saline solution (pH = 7.8)			

Figure 5.1 Color changes of polymer films coated with phenolic compounds from avocado byproduct extract. The coated films were reacted with silver nitrate solution for at least 24 hours.

5.1.2 DPPH• assay

5.1.2.1 Introduction

DPPH is a chromogen. This organic chemical is normally utilized to detect the antioxidant efficacy of phenolic compounds.

In order to evaluate the antioxidant property, aqueous DPPH• solution needs to be prepared by mixing DPPH powder into a solvent. When DPPH powder completely dissolves in the solvent, a purple color solution can be obtained indicating the formation of DPPH•. Then, phenolic compounds can be added into the DPPH• working solution to initiate the antioxidant activity. During this process, phenolic compounds locate DPPH• radicals in the solution, donate single electrons and/or hydrogen atoms to DPPH• radicals (Figure 5.2), and thus decrease the concentration of DPPH•. At the same time, the DPPH• solution gradually transforms to a yellow color, implying the end of the reaction. The color-change speed depends on the concentration of the phenolic compounds in the DPPH• working solution. The decrease in DPPH• concentration can be detected in a UV-Vis spectrophotometer at 517 nm and quantified by Equation 5.1.

$$\text{DPPH• inhibition capacity (\%)} = \frac{\text{Abs}_{\text{sample}_{t_0}} - \text{Abs}_{\text{sample}_{t_i}}}{\text{Abs}_{\text{sample}_{t_0}}} \times 100$$

(Equation 5.1)

Where

$Abs_{sample_{t_0}}$ = The absorbance reading of DPPH• working solutions used for antioxidant test of coated films at time t_0

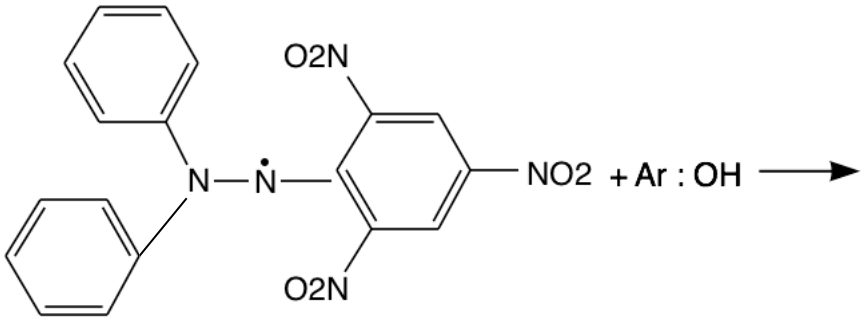
$Abs_{sample_{t_i}}$ = The absorbance reading of DPPH• working solutions used for antioxidant test of coated films at time t_i, $i = 1,2 \ldots t$

To acquire an accurate understanding about the DPPH• inhibition ability of various phenolic compounds, researchers normally introduce and calculate EC_{50}. EC_{50} refers to the effective concentration required for antioxidants to reduce 50% of the free radicals in a working solution. In order to determine this value, the DPPH• assay needs to be performed to establish a correlation between the total quantity of DPPH• radicals in the working solution (the UV-Vis absorbance readings of a DPPH• working solution) and the concentration of phenolic compound(s) added in the DPPH• working solution. Once the correlation is confirmed, EC_{50} can be determined by fitting the experimental results in different mathematical models, including logistic, Boltzmann sigmoidal and dose-response models (Suriyatem et al. 2017).

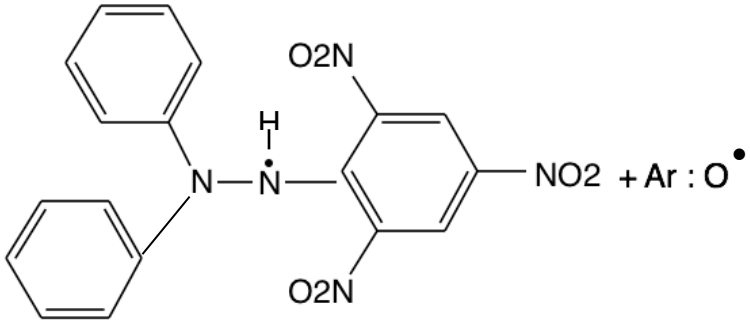
For this research, coated sample films with the same surface area were tested for the evaluation of antioxidant efficiency. The goal was to understand the correlation between the total quantity of DPPH• radicals in the working solution and the effectiveness of the phenolic coating layer in eliminating DPPH• radicals. In other words, rather than the phenolic compound

concentrations studied in other research, the reaction time used for DPPH• elimination in this research was the parameter of interest. Therefore, ET_{50} was introduced, referring to the effective time required for the phenolic coating layer to reduce 50% of the free radicals in a working solution.

The DPPH• assay is easy to prepare. It is an effective method to study the efficacy of phenolic compounds stabilizing free radicals. However, DPPH is insoluble in water (Stasko et al. 2007), the entire reaction process is time consuming, and it is expensive. These non-negligible factors limit its applications for various antioxidants in different environments.



DPPH•



DPPH-H

Where

Ar : OH is a phenolic compound

Ar : O• is a phenolic radical

Figure 5.2 Single electron donation reaction between a DPPH• radical and a phenolic compound.

5.1.2.2 Materials

DPPH powder was purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). Ethanol (200 proof) was purchased from VWR International (Radnor, PA, U.S.A.). These chemicals were of reagent grade.

Ethanolic seed extract was utilized to coat PP, PET, and LDPE films by applying the laccase assist coating (section 4.3.1) and alkaline saline assist coating (section 4.3.2) methods.

5.1.2.3 Methods

To make the DPPH• working solution, DPPH powder was dissolved in 95% aqueous ethanol (fatty food simulants) and 50% aqueous ethanol (simulating milk and products with high alcohol content) to obtain a concentration of 0.1 mM.

Then, a 2 × 1.5 cm sample film (PP, PET, or LDPE) with the phenolic coating layer was immersed into 1 mL of DPPH• working solution to initiate the antioxidant activity at room temperature. At each predetermined time interval, the immersed sample film was temporarily removed from the working solution, and the absorbance reading of the DPPH• working solution was taken at 517 nm in a Shimadzu UV-Vis spectrophotometer (Kyoto, Japan, model: UV-1800). After the measurement, the sample film was placed back in the working solution to allow further reaction. Light exposure was avoided during the entire electron donation reaction. The DPPH• working solution was freshly made every time before the analysis.

For comparison purposes, uncoated sample films immersed in DPPH• working solution were used as control samples and treated in the exact same way.

5.1.2.4 Results and discussion

5.1.2.4.1 Coating solution vs. antioxidant efficacy

Sample films coated by the two different solutions did not behave in the same way to reduce DPPH• radicals in the working solutions. As shown in Figures 5.3 and 5.4, the free radical reduction process of LDPE films coated in the alkaline saline solution (pH = 7.8) was rapid. For both DPPH• tests, the alkaline solution coated LDPE films stabilized around 86% of the free radicals within 5 hours. To eliminate the same amount of DPPH• radicals, the laccase solution coated LDPE film in 50% aqueous ethanol required 9 hours. After 48 hours, the laccase solution coated LDPE film in 95% aqueous ethanol reduced only 60% of the free radicals in the working solution.

Based on the ET_{50} values of the coated LDPE films in both 50% and 95% aqueous ethanol, the alkaline saline coating method was, again, suggested as a more effective approach than the laccase assist coating method. In 50% aqueous ethanol, the coated sample film used around 30 minutes to eliminate 50% of DPPH• radicals, which is at least 6 times faster than the laccase solution coated LDPE film. In 95% aqueous ethanol, the time difference increased to 93 times.

To further confirm the effectiveness of the alkaline saline solution, coated PET films were tested in 50% aqueous ethanol. As shown in Figure 5.5, the phenolic coating layer generated by the alkaline solution still displayed potent antioxidant ability. Similar to the alkaline solution coated LDPE films, the alkaline solution coated PET film presented a faster DPPH• reduction process. The ET_{50} of the laccase solution coated PET film was around 2.5 hours, which was 6.8 times longer than that of the alkaline solution coated PET film.

Based on the above analysis, the alkaline saline solution resulted in a more active and efficient coating to stabilize DPPH• radicals in 50% aqueous ethanol (milk and high alcohol content food simulant) and 95% aqueous ethanol (fatty food simulant). This result agreed with the indications from the previous silver nitrate test. Since DPPH powder is insoluble in water, another assay was desired to understand whether the same experimental outcome could be obtained in a water-based food simulant (10% aqueous ethanol).

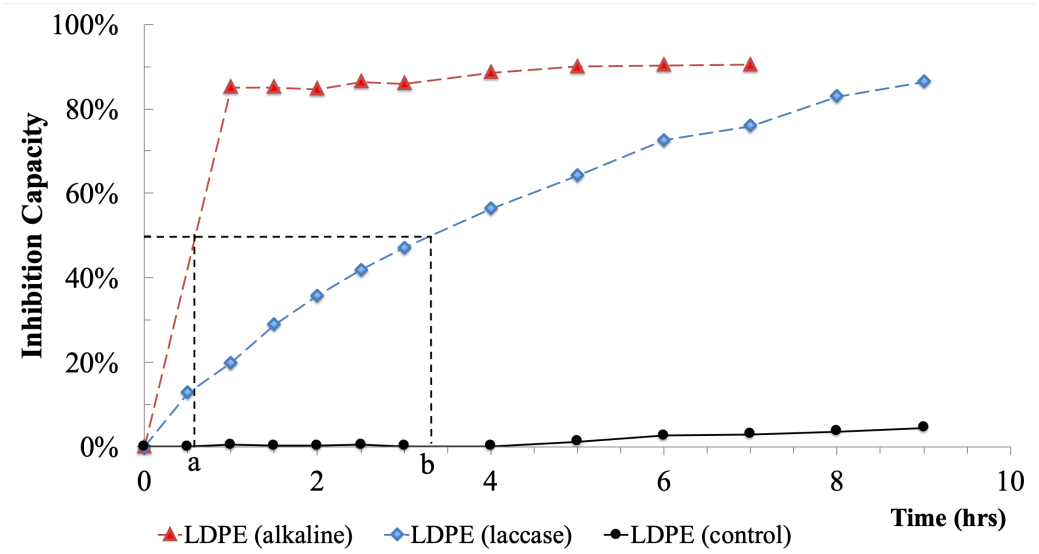


Figure 5.3 Antioxidant activity of LDPE films coated in laccase assist (pH = 5) and alkaline saline (pH = 7.8) solutions. The DPPH• solvent was diluted with 50% aqueous ethanol. Points a and b are estimates of the ET_{50} of LDPE (alkaline) and LDPE (laccase) respectively.

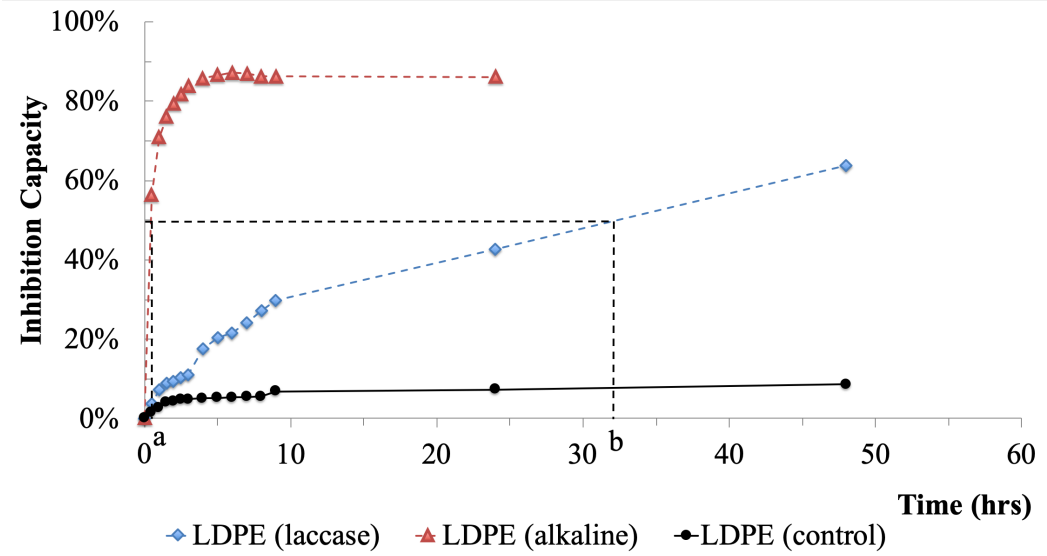


Figure 5.4 Antioxidant activity of LDPE films coated in laccase assist (pH = 5) and alkaline saline (pH = 7.8) solutions. The DPPH• solvent was diluted with 95% aqueous ethanol. Points a and b are estimates of the ET_{50} of LDPE (alkaline) and LDPE (laccase) respectively.

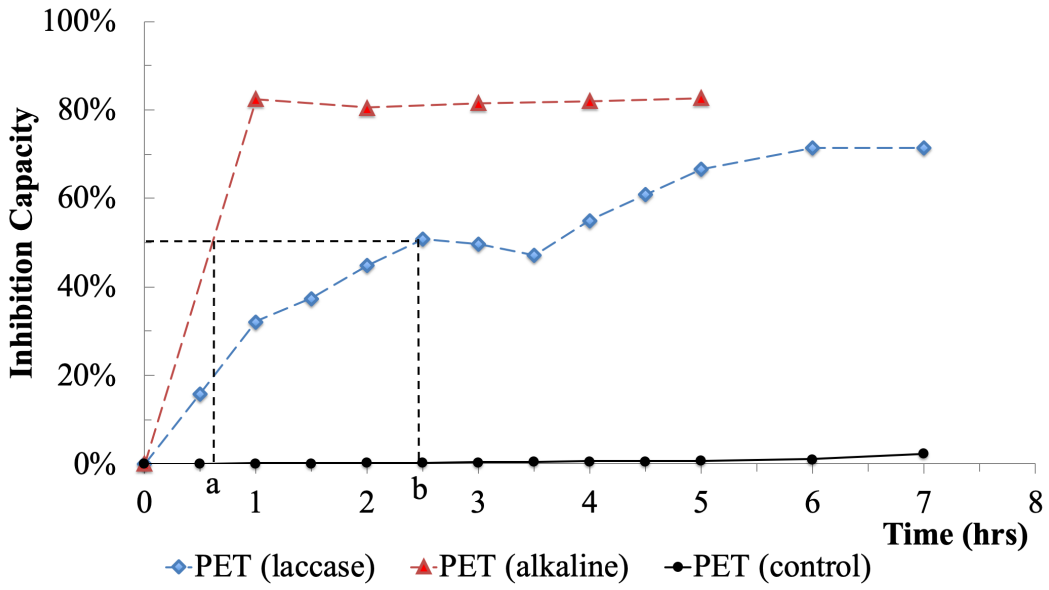


Figure 5.5 Antioxidant activity of PET films coated in laccase assist (pH = 5) and alkaline saline (pH = 7.8) solutions. The DPPH• solvent was diluted with 50% aqueous ethanol. Points a and b are estimates of the ET_{50} of LDPE (alkaline) and LDPE (laccase) respectively.

5.1.2.4.2 Plastic substrates vs. antioxidant efficacy

It is worth mentioning that there was no apparent time difference between the estimated ET_{50} of alkaline solution coated PP, LDPE and PET films in 50% aqueous ethanol (see Figure 5.6). These coated sample films exhibited similar antioxidant behavior. Within the first hour, all the alkaline solution coated sample films eliminated 80% of the DPPH• radicals in the working solution.

A small time difference was noticed between the estimated ET_{50} of laccase solution coated LDPE and PET films. The ET_{50} of laccase solution coated PET film was estimated at 2.5 hours, which was 0.8 hour faster than the LDPE film coated in the same type of solution.

These experimental outcomes suggested that the polymer substrates did not exert a major influence on the antioxidant efficacy of the phenolic coating.

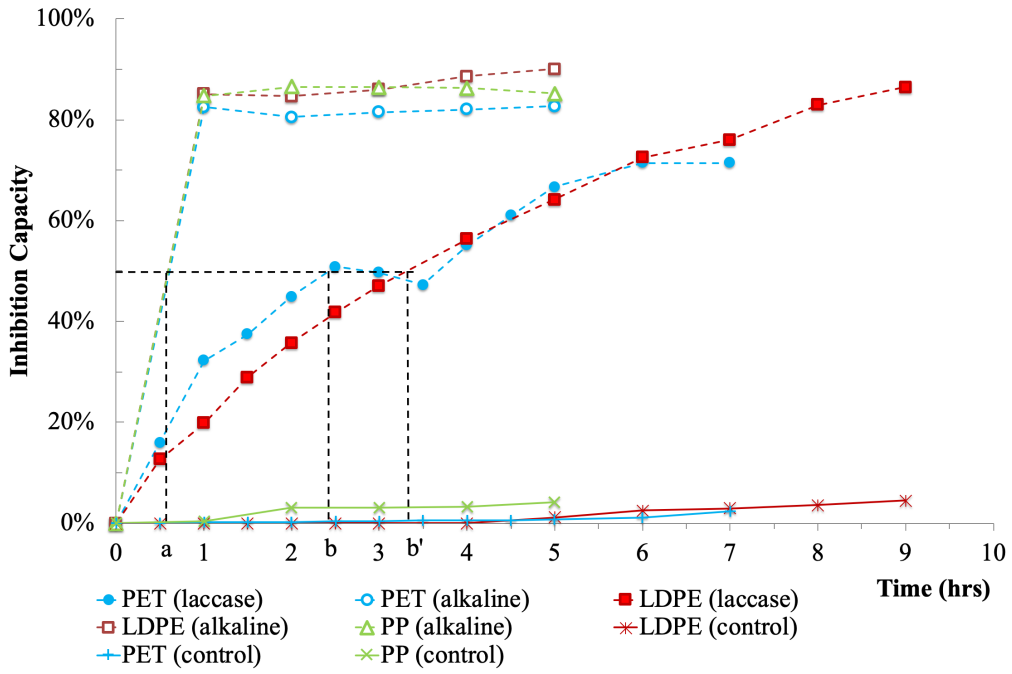


Figure 5.6 Antioxidant activity of PET, LDPE and PP films coated in laccase assist (pH = 5) and alkaline saline (pH = 7.8) solutions. The DPPH• solvent was diluted with 50% aqueous ethanol. Point a is the estimate of ET_{50} of LDPE (alkaline), PET (alkaline) and PP (alkaline). Points b and b' are estimates of the ET_{50} of PET (laccase) and LDPE (laccase) respectively.

5.1.2.4.3 Concentration of phenolic extract vs. antioxidant efficacy

In order to understand the correlation between the concentration of phenolic extract in the coating solution and the antioxidant efficacy of the coating layer, the alkaline solution coated PP films were tested. For the purpose of comparison, 12.5 mg/mL and 25 mg/mL ethanolic avocado seed extract were used in the coating process.

As shown in Figure 5.7, no significant time difference was observed between the free radical inhibition process of the two samples. The alkaline solution with doubled seed extract appeared to slightly reduce the estimated ET_{50} but the difference was small. Within 1 hour, both coated films reduced DPPH• concentration in the working solution by at least 70%.

Based on this finding, the excess phenolic content in the coating solution might not attach to the film surface during the coating process. Alternatively, provided the coating duration and phenolic content in the coating solution were sufficient, the antioxidant efficiency of the coating layer might be determined by substrate surface area rather than the amount of phenolic content in the coating solution. Further evaluation is desired to understand the minimum coating duration and phenolic concentration in the coating solution to obtain an adequate coating layer on different surface areas.

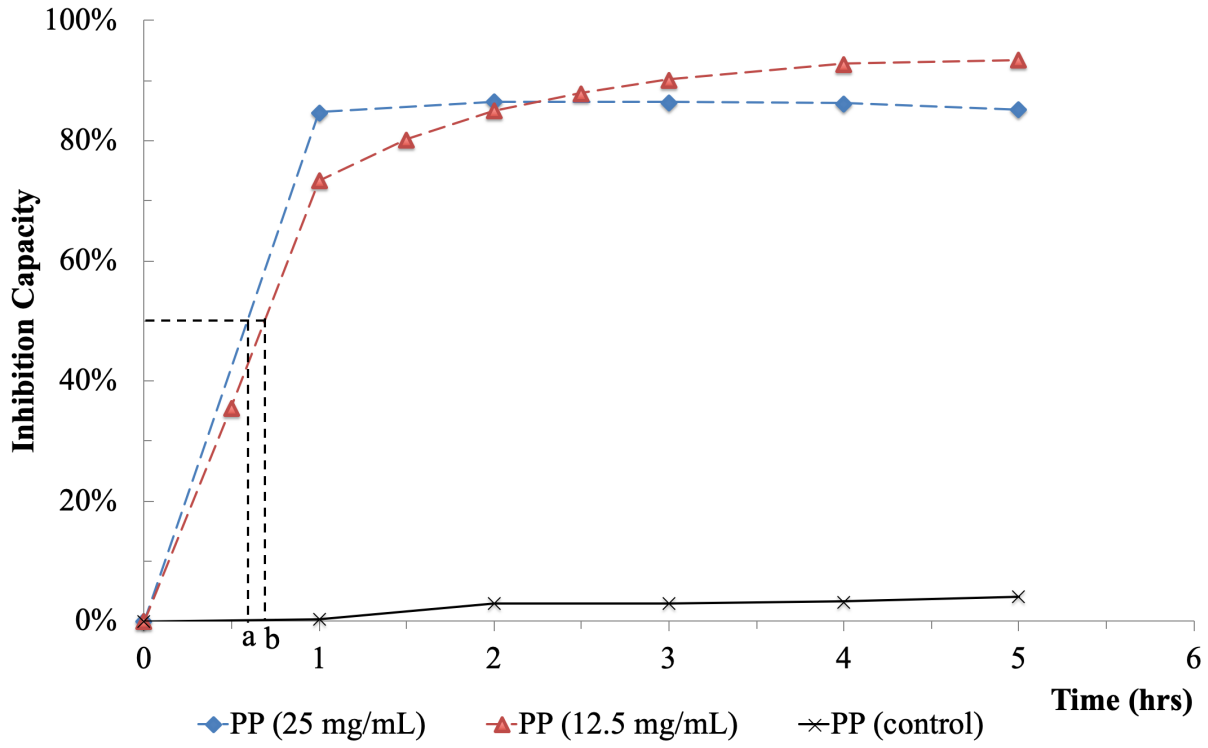


Figure 5.7 Antioxidant activity of PP films coated in solutions with different extract concentrations. The DPPH• solvent was diluted with 50% aqueous ethanol. The films were coated in alkaline saline (pH = 7.8) solution. Points a and b are estimates of the ET_{50} of PP films coated in solutions with 25mg/mL and 12.5mg/mL avocado seed extract.

In conclusion, the DPPH• assay was employed at the secondary experimental stage to evaluate potential influences of the coating environment, plastic substrates and the concentration of phenolic extract in the coating solution on the antioxidant effectiveness of the phenolic coating layer. As was indicated by the silver nitrate test, the sample films coated in alkaline saline solution (pH = 7.8) presented greater antioxidant effectiveness in 50% aqueous ethanol

(milk and high alcohol content food simulant) and 95% aqueous ethanol (fatty food simulant). While testing the impact of plastic substrates and phenolic concentration in the coating solution on the free radical reduction process, significant variation was not observed. It seemed that the antioxidant efficiency of the coating layer was not linearly correlated to the concentration of phenolic extract in the coating solution. As long as enough phenolic content could be provided for substrate surface attachment, excess phenolic content in the coating solution did not appear to create a more potent coating layer. Further experiments are desired to understand the minimum coating duration and phenolic concentration in the coating solution to obtain an effective coating layer on various surface areas.

In addition, another assay is desired to understand the antioxidant efficiency of the coating layer in water-based food simulant (10% aqueous ethanol) as DPPH powder is insoluble in water.

5.1.3 ABTS$\bullet^+$ assay

5.1.3.1 Introduction

ABTS is another type of chromogen. It is a common chemical used for understanding the dynamic process of the antioxidant activity of phenolic compounds.

In order to evaluate the antioxidant property, aqueous ABTS solution needs to be oxidized by potassium persulfate ($K_2S_2O_8$) first to obtain ABTS$\bullet^+$ solution (Figure 5.8). During

the oxidation process, the solution gradually changes its color to dark blue-green indicating the formation of ABTS•$^{+}$. After that, a small portion of diluted ABTS•$^{+}$ solution is used to evaluate the antioxidant ability of phenolic compounds by mixing it with the phytochemicals (Figure 5.9). During this process, phenolic compounds locate ABTS•$^{+}$ radicals in the solution, transfer their hydrogen atoms to ABTS•$^{+}$ radicals (hydrogen abstraction of antioxidants), and thus decrease the concentration of ABTS•$^{+}$ in the working solution. Under this circumstance, the ABTS•$^{+}$ working solution gradually loses its blue-green color. The color-change speed depends on the concentration of the phenolic compounds mixed with the diluted ABTS•$^{+}$ solution. The decrease of ABTS•$^{+}$ concentration can be detected in a UV-Vis spectrophotometer at 734 nm and quantified by Equation 5.2.

$$\text{ABTS}\bullet^{+}\ \text{inhibition capacity}\ (\%) = \frac{\text{Abs}_{\text{sample}_{t_0}} - \text{Abs}_{\text{sample}_{t_i}}}{\text{Abs}_{\text{sample}_{t_0}}} \times 100$$

(Equation 5.2)

Where

$Abs_{sample_{t_0}}$ = The absorbance reading of ABTS•$^{+}$ working solutions used for antioxidant test of coated films at time t_0

$Abs_{sample_{t_i}}$ = The absorbance reading of ABTS•$^{+}$ working solutions used for antioxidant test reacted with coated films at time t_i, $i = 1,2 \ldots t$

ABTS$\bullet^+$ radicals react with antioxidants rapidly. Unlike DPPH$\bullet$, this free radical can be dissolved in both aqueous and organic solvents, and the pH value of the working solution does not negatively impact the existence of ABTS$\bullet^+$ (Shalaby 2013). All these aforementioned facts enable ABTS$\bullet^+$ to be widely utilized as an efficient agent to evaluate the antioxidant effectiveness of phenolic compounds.

For this test, coated sample films with the same surface area were tested for the evaluation of antioxidant efficiency. The aim was to understand the correlation between the total quantity of ABTS$\bullet^+$ radicals in the working solution and the effective time the phenolic coating layer used for ABTS$\bullet^+$ elimination. To acquire an accurate understanding about the ABTS$\bullet^+$ inhibition ability of the phenolic coating layer, and conduct valid comparisons among the ABTS$\bullet^+$ tests, ET_{50} was introduced again. This term refers to the effective time required for the phenolic coating layer to reduce 50% of the free radicals in a working solution.

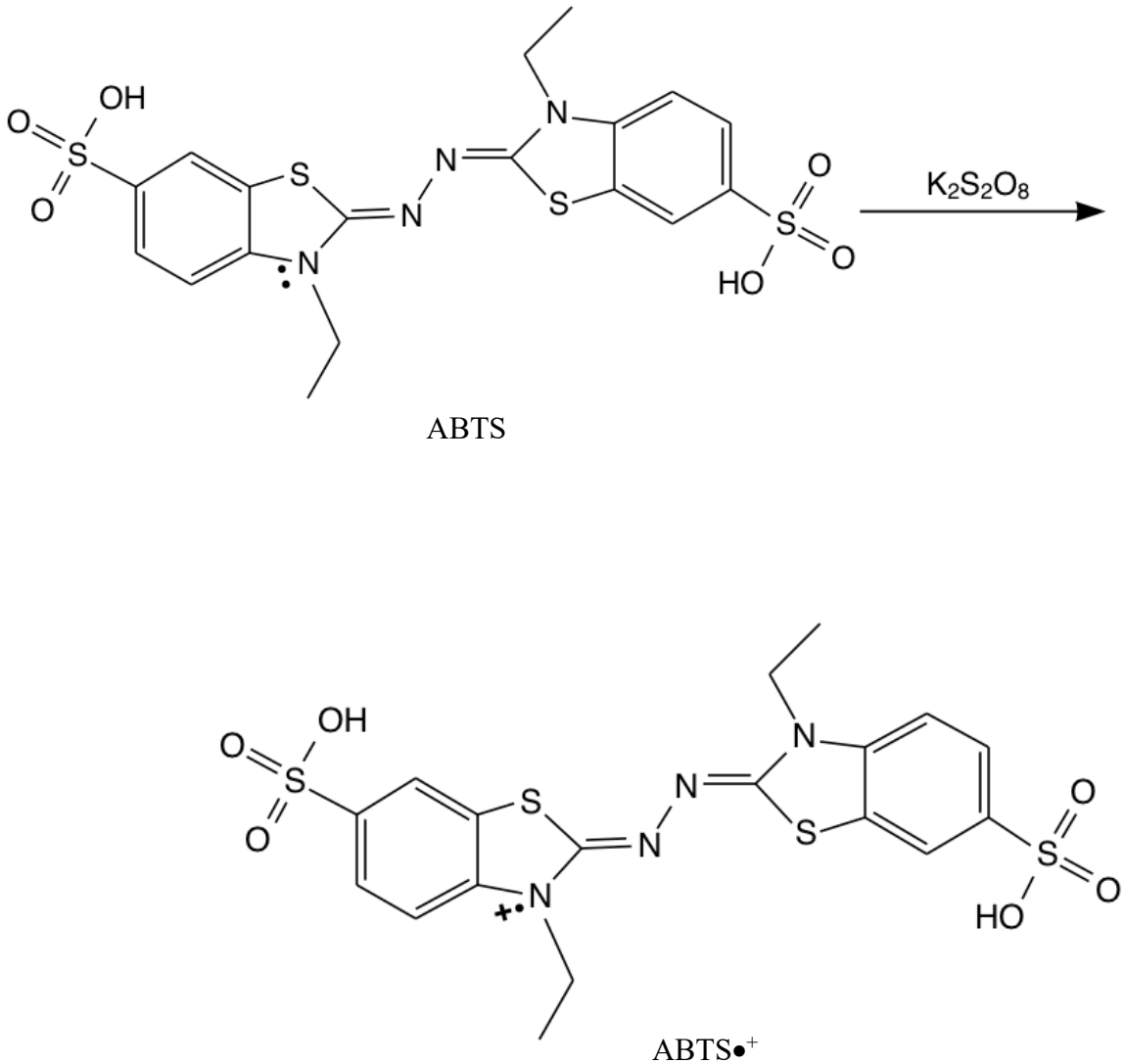


Figure 5.8 Oxidation reaction of ABTS with potassium persulfate to generate ABTS•$^{+}$ radicals.

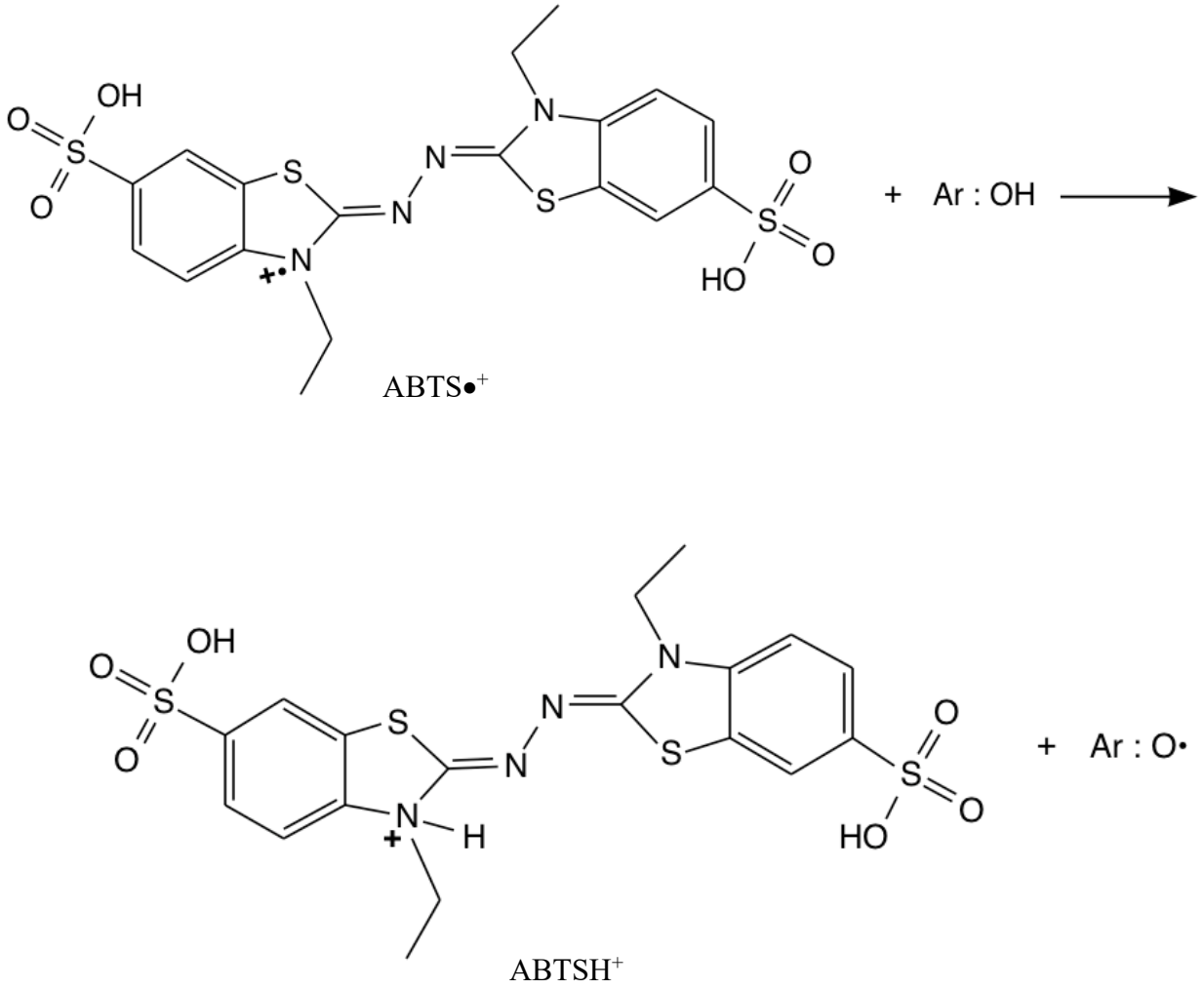


Figure 5.9 Hydrogen abstraction reaction of ABTS•$^{+}$ with a phenolic compound.

5.1.3.2 Materials

ABTS and potassium persulfate powders were purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). All the chemicals were of reagent grade. Before the experiment, ABTS powder was stored under refrigeration at 4 °C and protected from light until use.

Both ethanolic and acetonic extracts of avocado seed and peel were utilized to coat PP, PET, and LDPE films by applying the laccase assist coating (section 4.3.1) and alkaline saline assist coating (section 4.3.2) methods.

5.1.3.3 Methods

ABTS$\bullet^+$ stock solution was made by mixing 7 mM aqueous ABTS solution with 2.45 mM aqueous potassium persulfate solution. The ratio of V_{ABTS} to $V_{K_2S_2O_8}$ was 1:1. The mixture was then stored in a dark bottle and dark room at room temperature for 16 hours to allow completion of the oxidation reaction between ABTS and potassium persulfate.

To make the ABTS$\bullet^+$ working solution, 1 mL ABTS$\bullet^+$ stock solution was diluted with around 60 mL 95% aqueous ethanol (fatty food simulant), 50% aqueous ethanol (simulant of high alcohol containing products and milk), or 10% aqueous ethanol (simulant of water-based food) to obtain an absorbance of 0.7 ± 0.05 at 734 nm in a Shimadzu UV-Vis spectrophotometer (Kyoto, Japan, model: UV-1800).

After that, a 2 × 1.5 cm sample film (PP, PET, or LDPE) with the phenolic coating layer was immersed into 1 mL of ABTS$\bullet^+$ working solution to initiate the antioxidant activity at room temperature. Every 6 minutes, the immersed sample film was temporarily removed from the working solution, and the absorbance reading of the working solution was taken at 734 nm in the UV-Vis spectrophotometer. After the measurement, the sample film was placed back in the

working solution to allow further reaction. Light exposure was avoided during the entire hydrogen abstraction reaction. The ABTS$\bullet^+$ working solution was freshly made every time before the analysis.

For comparison purposes, uncoated sample films immersed in ABTS$\bullet^+$ working solution were used as control samples and treated in the exact same way.

5.1.3.4 Results and discussion

5.1.3.4.1 Antioxidant efficacy of the phenolic extracts from avocado byproducts

In order to understand the antioxidant efficacy of the phenolic extracts from avocado byproducts, one drop (around 0.83 μl) of the acetonic peel or seed extract was directly added into 1mL ABTS$\bullet^+$ working solution (diluted with 95% aqueous ethanol) for evaluation. This screening test was conducted before evaluating the coated sample films.

Figure 5.10 presents the free radical reduction process of the peel and seed extracts. Even though $\frac{V_{\text{ABTS}\bullet+}}{V_{extract}} \approx 1200$, both peel and seed extracts exhibited potent antioxidant ability stabilizing 50% of ABTS$\bullet^+$ radicals within 1 minute. Compared with the seed extract, the peel extract was more powerful. The ET_{50} of the peel extract was 9.5 seconds, which was only 19% of the time the seed extract used to reach its ET_{50} point. In addition, at the end of the test (3 minutes), the peel extract had stabilized 100% of the free radicals in the working solution, whereas 40% of ABTS$\bullet^+$ radicals still remained in the other working solution. This experimental

outcome agreed with the phenolic quantification and identification results discussed in section 3.3. Based on the analysis result of the mass spectra (Table 3.1 and Figure 3.1), there were 22 out of 28 types of phenolic compounds available in both avocado peel and seed extracts. Among these phytochemicals, the concentrations of catechin isomer 2, procyanidin dimer B (I), 5-O-caffeoylquinic acid, procyanidin trimer 2 and procyanidin trimer 1 in the peel extract were significantly greater than that in the seed extract. These high content phenolic compounds enabled the peel extract to eliminate more ABTS$\bullet^{+}$ radicals in the working solution at a faster rate.

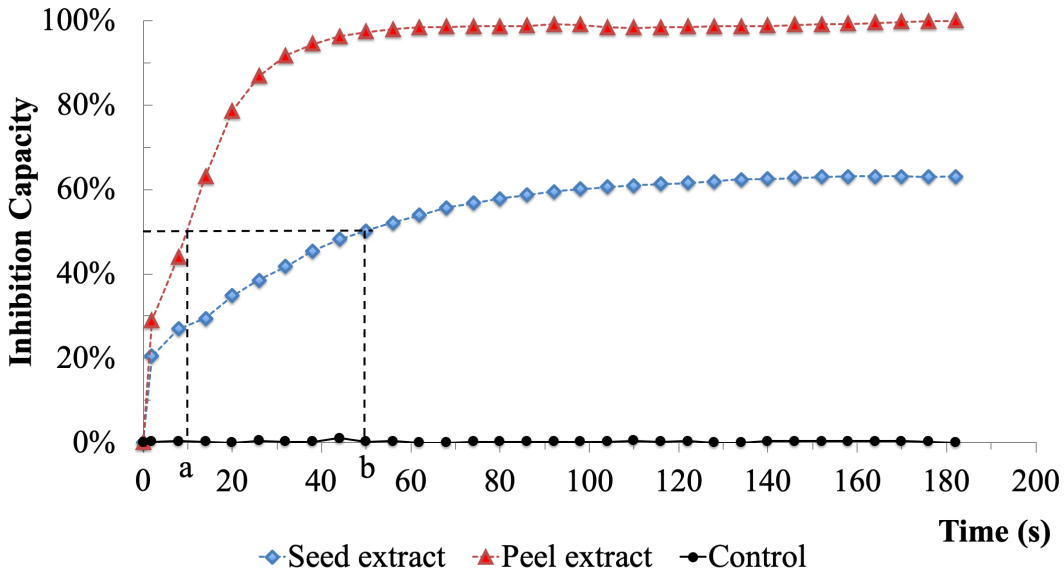


Figure 5.10 Antioxidant activity of acetonic phenolic extracts from avocado byproducts. Points a and b are the estimated ET_{50} of the peel and seed extract respectively. The ABTS$\bullet^{+}$ solvent was diluted with 95% aqueous ethanol.

5.1.3.4.2 Coating solutions vs. antioxidant efficacy

In order to confirm that the alkaline saline solution could provide a more powerful phenolic coating than the laccase assist solution, ethanolic seed extract was utilized again for this screening test.

As shown in Figure 5.11, the ABTS$\bullet^{+}$ evaluation result further confirmed that the alkaline solution coated sample film was more potent than the laccase solution coated polymer substrate. Within 18 minutes, the PP film coated by the alkaline solution completely eliminated ABTS$\bullet^{+}$ radicals in the working solution. During the same time period, the laccase solution coated PP film only reduced 30% of ABTS$\bullet^{+}$ radicals. After 90 minutes, 16% of ABTS$\bullet^{+}$ radicals still remained in the working solution used for testing the laccase solution coated PP film.

The free radical reduction process of ABTS$\bullet^{+}$ assay was rapid. Different from the long reaction time of DPPH$\bullet$ assay, the effective duration of ABTS$\bullet^{+}$ assay could be controlled within 2 hours. In comparing the free radical reduction process of alkaline solution coated polymer films (as shown in Figure 5.12), significant time difference was noticed. The coated films could stabilize ABTS$\bullet^{+}$ radicals in 30 minutes, whereas the DPPH$\bullet$ evaluation time was extended to 5 hours. After 5 hours, around 15% of DPPH$\bullet$ radicals still remained in the working solutions.

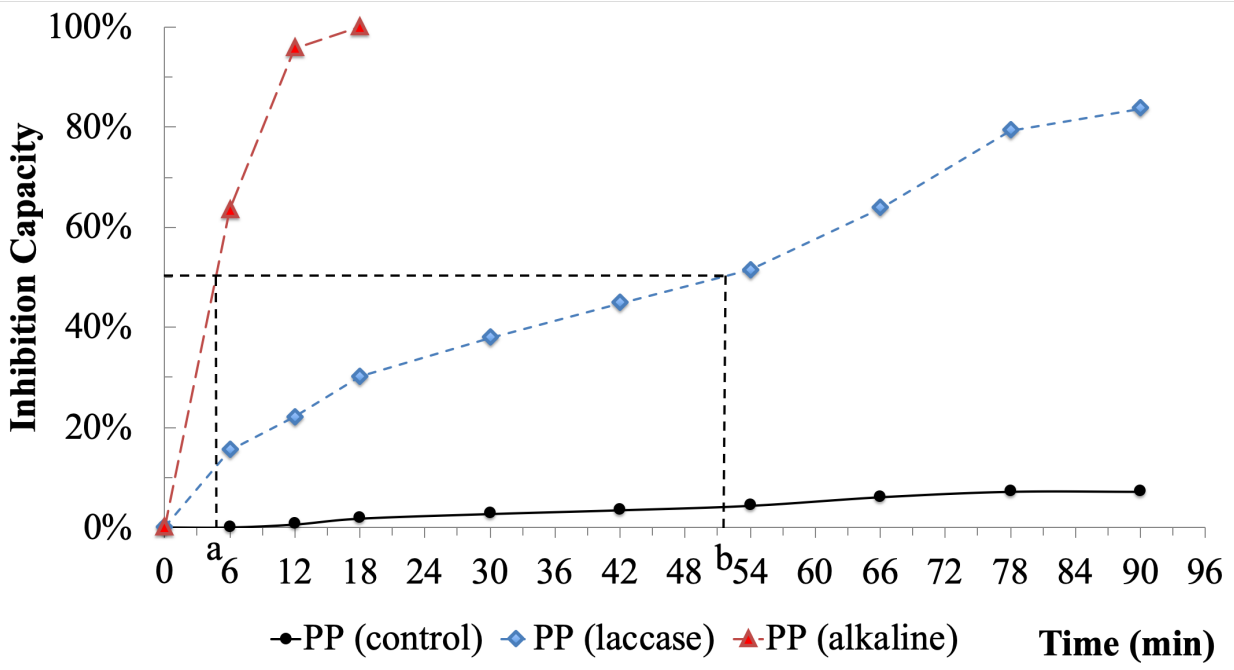


Figure 5.11 Antioxidant activity of PP films coated in laccase assist (pH = 5) and alkaline saline (pH = 7.8) solutions. The ABTS$\bullet^{+}$ solvent was diluted with 50% aqueous ethanol. Points a and b are estimated ET_{50} of PP (alkaline) and PP (laccase) respectively.

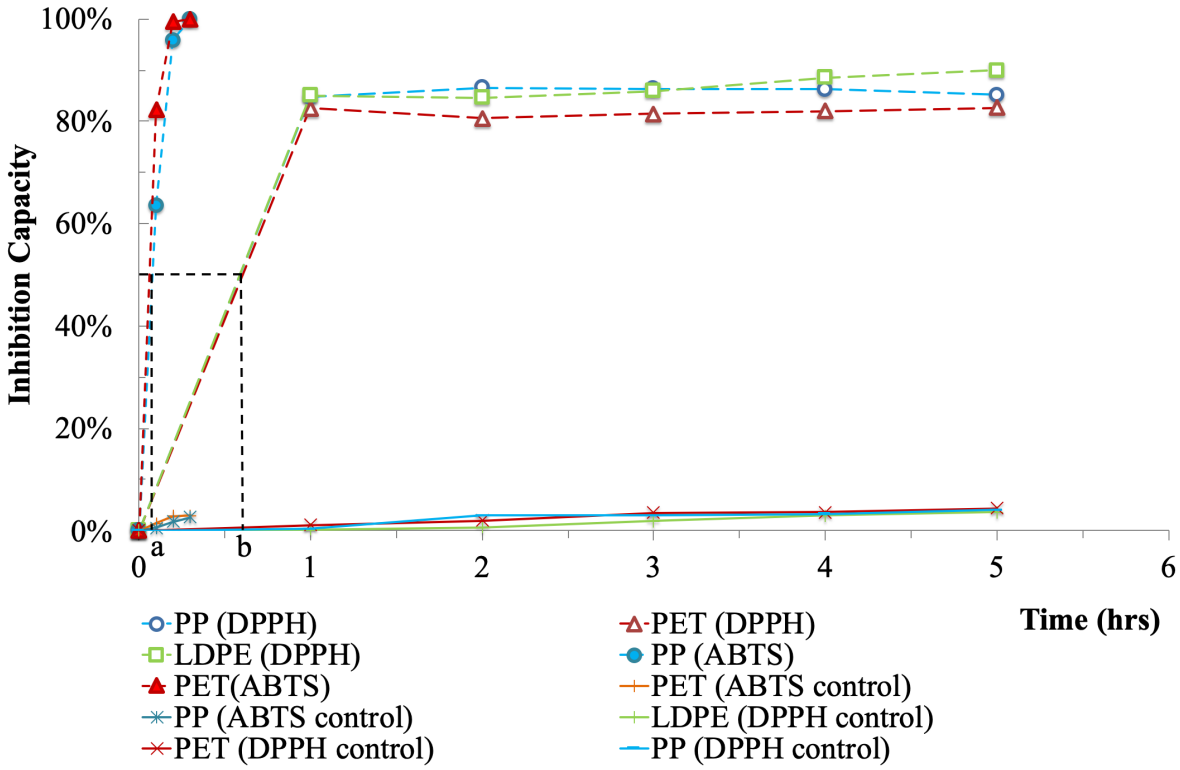


Figure 5.12 Antioxidant activity of PP, PET and LDPE films coated in alkaline saline (pH = 7.8) solutions. The ABTS•$^{+}$ and DPPH• solvents were diluted with 50% aqueous ethanol. Point a is the estimated ET_{50} of PP (ABTS) and PET (ABTS), and point b is the estimated ET_{50} of LDPE (DPPH), PET (DPPH) and PP (DPPH).

5.1.3.4.3 Plastic substrates vs. antioxidant efficacy

To examine the influence of plastic substrates on the antioxidant efficacy of the phenolic coating, ethanolic seed extract was used to coat PP and PET films in alkaline saline solution, and then immersed in 10% aqueous ethanol (water-based food simulant) containing ABTS•$^{+}$ radicals for a quick preliminary test. Based on Figure 5.13, it took nearly equal time for both films to

quench 50% of ABTS•$^{+}$ radicals in the solution. There was only a 0.4 minute gap between the estimated ET_{50} of the coated PET and PP films. This experimental result further supported the conclusion that polymer substrates did not act as a major factor influencing the antioxidant efficacy of the phenolic coating.

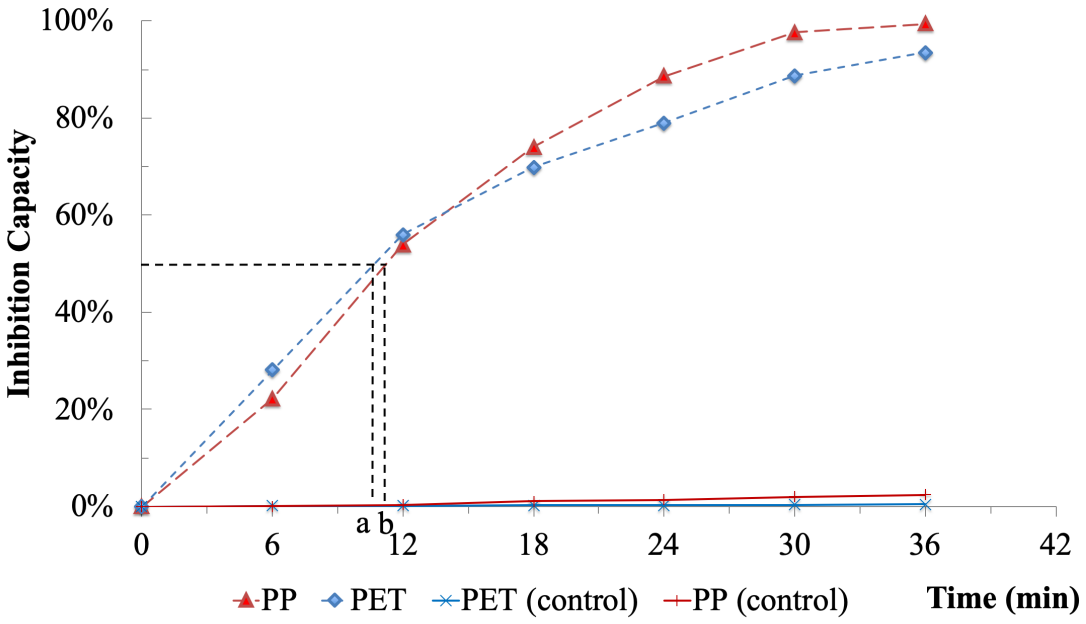


Figure 5.13 Antioxidant activity of PET and PP films coated with ethanolic seed extract. The films were coated in alkaline saline (pH = 7.8) solution. The ABTS•$^{+}$ solvent was diluted with 10% aqueous ethanol. Points a and b are the estimated ET_{50} of PET and PP respectively.

To sum up, the ABTS•$^{+}$ assay is a rapid testing method compared with the DPPH• assay. It was employed at the third experimental stage to evaluate the antioxidant efficacy of the avocado peel and seed extracts, and the potential influences of the coating environment and plastic substrates on the antioxidant effectiveness of the phenolic coating layer. In addition, the avocado peel and seed extracts were directly evaluated to understand their antioxidant potency to quench free radicals.

According to the analysis results, the high content phenolic compounds enabled the peel extract to eliminate more ABTS•$^{+}$ radicals in the working solution at a faster rate. Like what was implied from the silver nitrate test and DPPH• assay, the sample films coated in alkaline saline solution presented greater antioxidant effectiveness than the laccase solution coated polymer substrates. Polymer substrates did not play a crucial role in influencing the antioxidant efficacy of the phenolic coating.

Part II: Further Evaluation with Statistical Analysis

5.2 Introduction

In this part, coated PP, PET and LDPE films were tested in triplicate in order to a) further verify the influence of plastic substrates, b) analyze the potential effect of film storage time, and c) evaluate the impact of $\frac{W_{peel\ extract}}{W_{seed\ extract}}$ in the coating solution on the antioxidant efficiency of the phenolic coating.

Considering the fast reaction rate, the solubility in both aqueous and organic solvents, and the stability of the working solution in different pH environments, the ABTS$\bullet^{+}$ assay (section 5.1.3) was selected to evaluate coated PP, PET and LDPE films in 95% aqueous ethanol (fatty food simulant). Due to the potent coating efficacy evaluated in the previous silver nitrate, DPPH$\bullet$ and ABTS$\bullet^{+}$ tests, the alkaline saline method (section 4.3.2) was employed to coat acetonic seed and peel extracts on the plastic substrates.

As discussed in section 5.1.2.1, researchers could fit their experimental results to different mathematical models to evaluate the antioxidant efficiencies of various phenolic compounds, including the following logistic, Boltzmann sigmoidal and dose-response models (Suriyatem et al. 2017):

Logistic model $$y = \frac{A_1 - A_2}{1 + (\frac{x}{x_0})^{Hillslope}} + A_2$$

Boltzmann sigmoidal model $$y = \frac{A_1 - A_2}{1 + exp^{(\frac{x - x_0}{dx})}} + A_2$$

Dose-response model $$y' = \frac{100}{1 + 10^{[(x_0 - x) \times Hillslope]}}$$

Where

y = Absorbance readings of a free radical working solution in a UV-Vis spectrophotometer

y' = Normalized absorbance readings of a free radical working solution in a UV-Vis spectrophotometer. It varies from 0% to 100%

A_1 = The minimum plateau of a free-radical absorbance reading versus phenolic compound concentration curve

A_2 = The maximum plateau of a free-radical absorbance reading versus phenolic compound concentration curve

x = Logarithm of phenolic compound concentration

x_0 = The median value of x or $LogEC_{50}$

dx = Time constant

Hillslope = The slope or steepness of a free-radical absorbance reading versus phenolic compound concentration curve

For this research, the goal was to understand the correlation between the total quantity of free radicals in a working solution and the reaction time the phenolic coating used for free radical elimination. Therefore, the logarithm of reaction time should be used to estimate the antioxidant efficacy of the phenolic coating rather than the logarithm of phenolic compound concentration, x. The x_0 estimated by the above models, in this case, should be $LogET_{50}$, referring to the logarithm of the effective time required for the phenolic coating layer to reduce 50% of free radicals in a working solution.

Considering the minimum plateau of a free radical quantity versus reaction time curve was 0 for this research, and the y value estimated by the Logistic model would be 0 when the reaction time was 1 minute, which could not reflect the real absorbance reading of a free radical working solution, the modified Boltzmann sigmoidal (Equation 5.3) and dose-response (Equation 5.4) models were finally selected to analyze the phenolic coating.

For the statistical analysis, IBM® SPSS (SPSS Inc., Chicago, IL, USA) and JMP Pro 14 (SAS Institute Inc., Cary, NC, USA) programs were utilized. Appendix B includes the analysis results regarding the goodness of fit and fitted regression equations estimated by the modified mathematical models.

Modified Boltzmann sigmoidal model

$$y = \frac{-A'_2}{1 + exp^{\left(\frac{t-t_0}{Slope}\right)}} + A'_2$$

(Equation 5.3)

Modified dose-response model

$$IC\ (\%) = \frac{100}{1 + 10^{[(t_0 - t) \times Slope]}}$$

(Equation 5.4)

Where

IC = The inhibition capacity of the antioxidant layer. This value is normalized. It varies from 0% to 100%

A'_2 = The maximum plateau of a free radical quantity versus reaction time curve

t_0 = $LogET_{50}$

t = Logarithm of reaction time

Slope = The steepness of an inhibition capacity curve

5.2.1 Plastic substrates vs. antioxidant efficacy

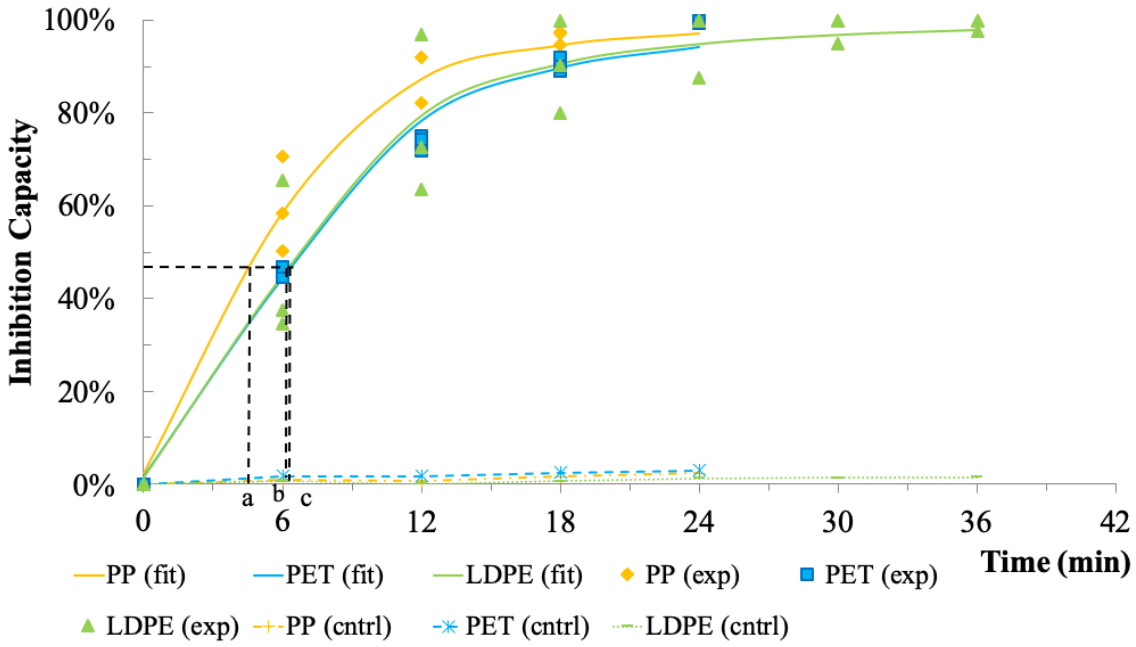
As shown in Figures 5.14 and 5.15, the coated PP, LDPE and PET films exhibit a similar antioxidant process to stabilize $ABTS^{\bullet+}$ radicals. These experimental outcomes agreed with the testing results obtained from the previous DPPH• assay (section 5.1.2.4.2) and $ABTS^{\bullet+}$ assay

(section 5.1.3.4.3). In other words, these data further supported that the polymer substrates did not exert a major influence on the antioxidant efficiency of the phenolic coating. Within 18 minutes, all the coated sample films eliminated at least 90% of ABTS$\bullet^{+}$ radicals in the working solution. The coated PP and PET films required comparatively less time than the coated LDPE films, as shown in both figures, to completely eliminate ABTS$\bullet^{+}$ radicals in the working solutions. However, the differences were not statistically significant.

There were also no significant differences in the estimated ET_{50} of the coated polymer films. Based on the dose-response and Boltzmann sigmoidal results in Figures 5.14 and Table 5.1, the PET and LDPE films coated with the seed extract used almost the same amount of time to stabilize 50% of ABTS$\bullet^{+}$ radicals, which was around 1.5-minute slower than the coated PP films. As for the polymer films coated with the peel extract (Figure 5.15 and Table 5.2), the similar antioxidant behavior allows them to reach their ET_{50} points with at most 1.1-minute time differences.

To understand the antioxidant efficiencies of the coated films at each time interval, another statistical analysis was performed. Based on Table 5.3 and 5.4, the phenolic coating on the PET, LDPE and PP films exhibited the same level of antioxidant efficacy during the same time interval. No statistically significant differences were found.

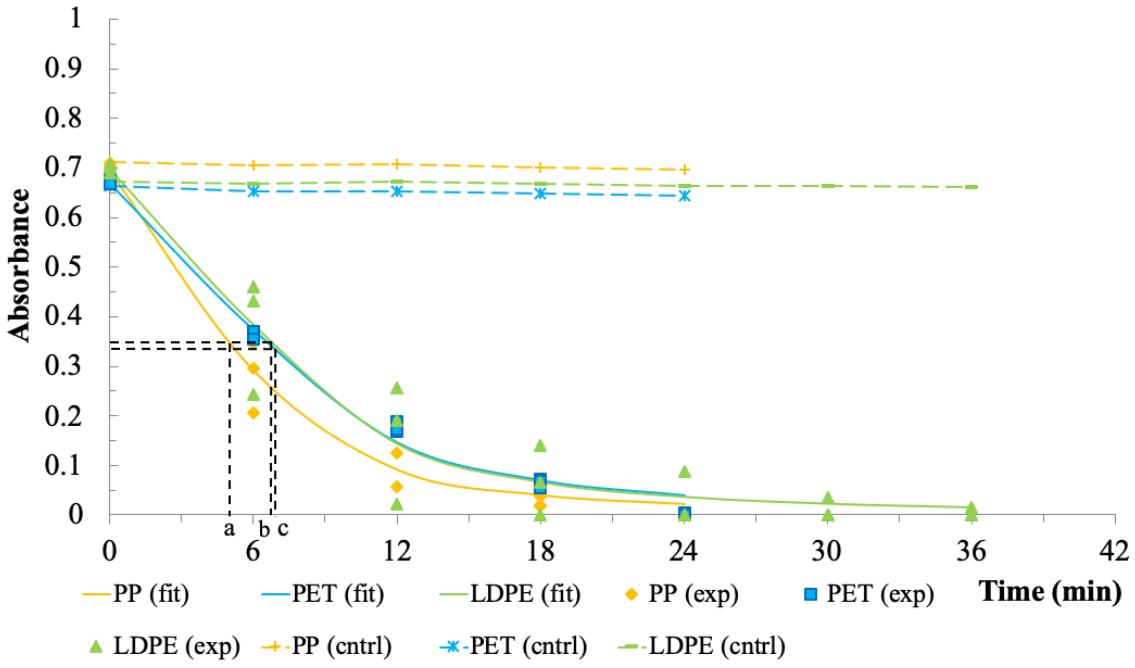
It is worth mentioning that the antioxidant efficiency of the coated LDPE films varied over a greater range (standard deviations) than the coated PP and PET films. Within the first 18 minutes of the ABTS•$^{+}$ test, the standard deviation of the antioxidant efficiency of the LDPE films increased to 19.16%. Possibly, phenolic residues, after the coating process, were attached to some spots of the film surface or edges and were not flushed away. These excessive phenolic compounds may have resulted in the large variations of the antioxidant efficiency.



A. Dose-response curves

Figure 5.14 Antioxidant activity of PP, PET and LDPE coated with acetonic seed extract. The films were coated in alkaline saline (pH = 7.8) solution. The ABTS•$^{+}$ solvent was diluted with 95% aqueous ethanol. Points a, b and c are the estimated ET_{50} of PP, LDPE and PET respectively.

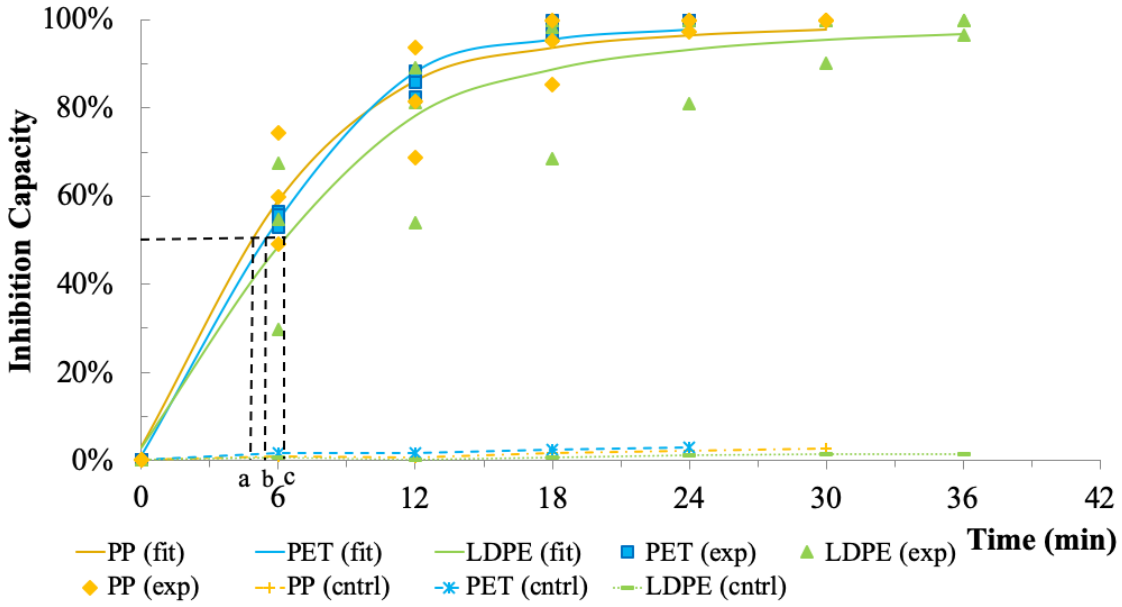
Figure 5.14 (cont'd)



B. Boltzmann sigmoidal curves

Table 5.1 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the plastic substrates vs. antioxidant efficacy test. $ABTS^{\bullet+}$ assay was applied to evaluate polymer films coated with the avocado seed extract.

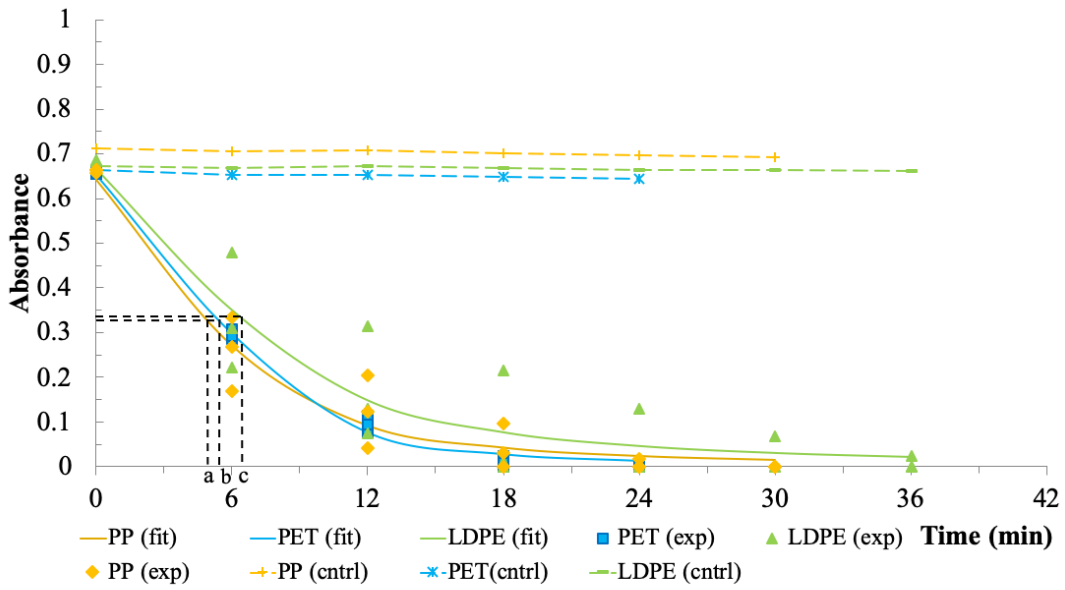
	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
PP	5.17 ± 1.07	5.10 ± 1.07	0.977	0.978	0.060	0.041
PET	6.66 ± 1.04	6.57 ± 1.04	0.991	0.991	0.038	0.025
LDPE	6.56 ± 1.09	6.47 ± 1.09	0.941	0.942	0.090	0.063



A. Dose-response curves

Figure 5.15 Antioxidant activity of PP, PET and LDPE coated with acetonic peel extract. The films were coated in alkaline saline (pH = 7.8) solution. The ABTS$\bullet^{+}$ solvent was diluted with 95% aqueous ethanol. Points a, b and c are the estimated ET_{50} of PP, PET and LDPE respectively.

Figure 5.15 (cont'd)



B. Boltzmann sigmoidal curves

Table 5.2 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the plastic substrates vs. antioxidant efficacy test. $ABTS^{\bullet+}$ assay was applied to evaluate polymer films coated with the avocado peel extract.

	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
PP	5.05 ± 1.10	5.03 ± 1.10	0.961	0.962	0.075	0.049
PET	5.60 ± 1.03	5.58 ± 1.03	0.996	0.996	0.026	0.017
LDPE	6.18 ± 1.13	6.13 ± 1.13	0.904	0.904	0.113	0.077

Table 5.3 Antioxidant efficiency of coated PET, LDPE and PP films at different time intervals. The sample films were coated with acetonic seed extract in alkaline saline (pH = 7.8) solution. ABTS$\bullet^{+}$ assay was selected as the evaluation method.

Time (min)	Inhibition Capacity %		
	PET	**LDPE**	**PP**
0	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
6	46.07 ± 1.19%[a]	45.87 ± 17.15%[a]	59.76 ± 10.28%[a]
12	73.77 ± 1.67%[a]	77.67 ± 17.34%[a]	83.06 ± 8.52%[a]
18	90.43 ± 1.50%[a]	90.10 ± 10.05% [a]	96.48 ± 1.56%[a]
24	99.77 ± 0.40%[a]	95.87 ± 7.16% [a]	99.90 ± 0.17%[a]
30		98.30 ± 2.94%	
36		99.23 ± 1.33%	

The inhibition capacity % is presented as mean value ± standard deviation

Values with the same superscript indicate no statistically significant difference among the antioxidant effectiveness of the PET, LDPE and PP films during the same time interval

Table 5.4 Antioxidant efficiency of coated PET, LDPE and PP films at different time intervals. The sample films were coated with acetonic peel extract in alkaline saline (pH = 7.8) solution. ABTS$\bullet^{+}$ assay was selected as the evaluation method.

Time (min)	Inhibition Capacity %		
	PET	**LDPE**	**PP**
0	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
6	55.12 ± 1.84%[a]	50.63 ± 19.16% [a]	61.08 ± 12.65%[a]
12	85.55 ± 2.83% [a]	74.76 ± 18.39% [a]	81.35 ± 12.51%[a]
18	98.33 ± 1.85% [a]	88.97 ± 17.73% [a]	93.57 ± 7.46%[a]
24	100.00 ± 0.00% [a]	93.66 ± 10.86% [a]	99.14 ± 1.49%[a]
30		96.73 ± 5.66%	100.00 ± 0.00%
36		98.88 ± 1.94%	

The inhibition capacity % is presented as mean value ± standard deviation

Values with the same superscript indicate no statistically significant difference among the antioxidant effectiveness of the PP, PET and LDPE films during the same time interval

5.2.2 Film storage time vs. antioxidant efficacy

After the coating process, storage time could be a main factor weakening the antioxidant efficacy of coated plastic substrates. In order to understand the variance between the antioxidant efficacy of coated films with different storage times, acetonic phenolic extracts from avocado seed and peel were used to coat PP films. Then, one group of the coated films was tested for their free radical reduction ability within 36 hours. Another group of films was evaluated after 6 days of storage time at room temperature (tested on day 7). $ABTS^{\bullet+}$ stock solution diluted with 95% aqueous ethanol was used for the analysis.

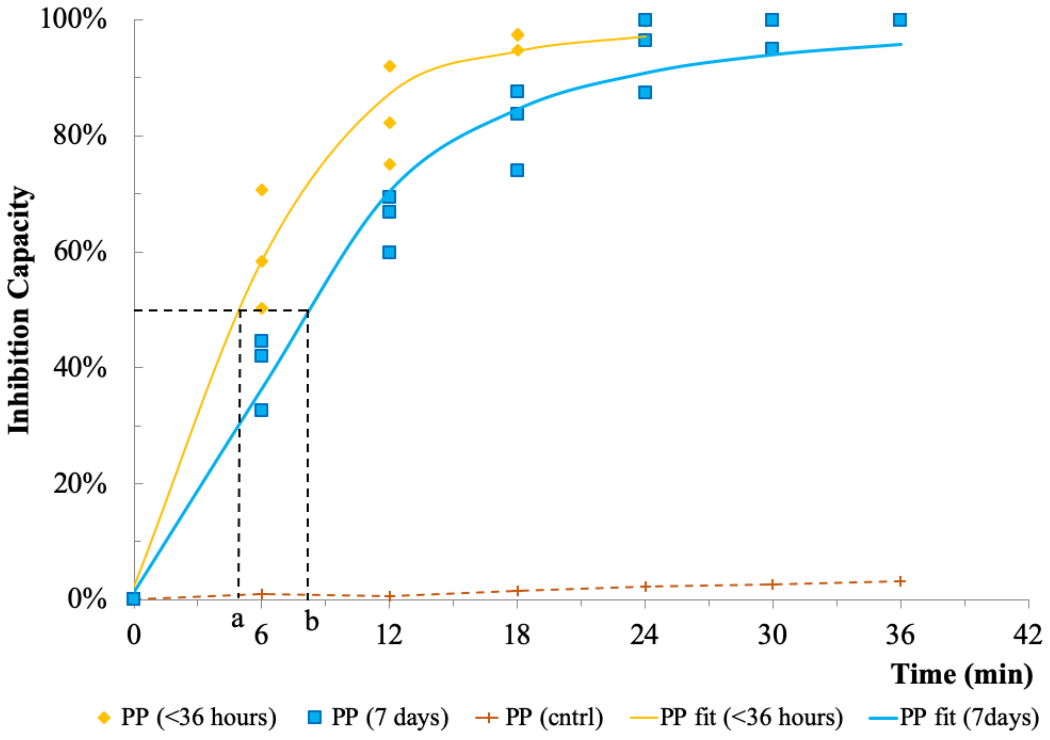
Figures 5.16 and 5.17 illustrate the antioxidant process of the coated PP films with different storage times. It is clear that the sample films tested on day 7 exhibited a comparatively slower rate to reduce $ABTS^{\bullet+}$ radicals in the working solution. However, the difference was eliminated after 18 minutes of reaction for the films coated with the peel extract.

For the ET_{50} estimated by the dose-response and Boltzmann models, the time difference between the substrates coated with the seed extract was approximately 2.7 minutes (Table 5.5), and that between the PP films coated with the peel extract was reduced to around 2 minutes (Table 5.6).

To understand the antioxidant efficiencies of the coated PP films at each time interval, a statistical analysis was performed. As shown in Table 5.7, the aforementioned differences

between the free radical reduction rates of the coated PP films with different storage times resulted in statistically significant differences at some time points. For the PP films coated with the avocado seed extract, significant differences were noticed within the first 18 minutes of the experiment. When the inhibition capacity of PP (7 days) increased to more than 90% at 24 minutes, the statistically significant difference no longer existed. For the PP films coated with the avocado peel extract, a statistically significant difference was noticed within the first 6 minutes of the $ABTS^{\bullet+}$ test. However, after 6 minutes of the antioxidant reaction, the difference was eliminated.

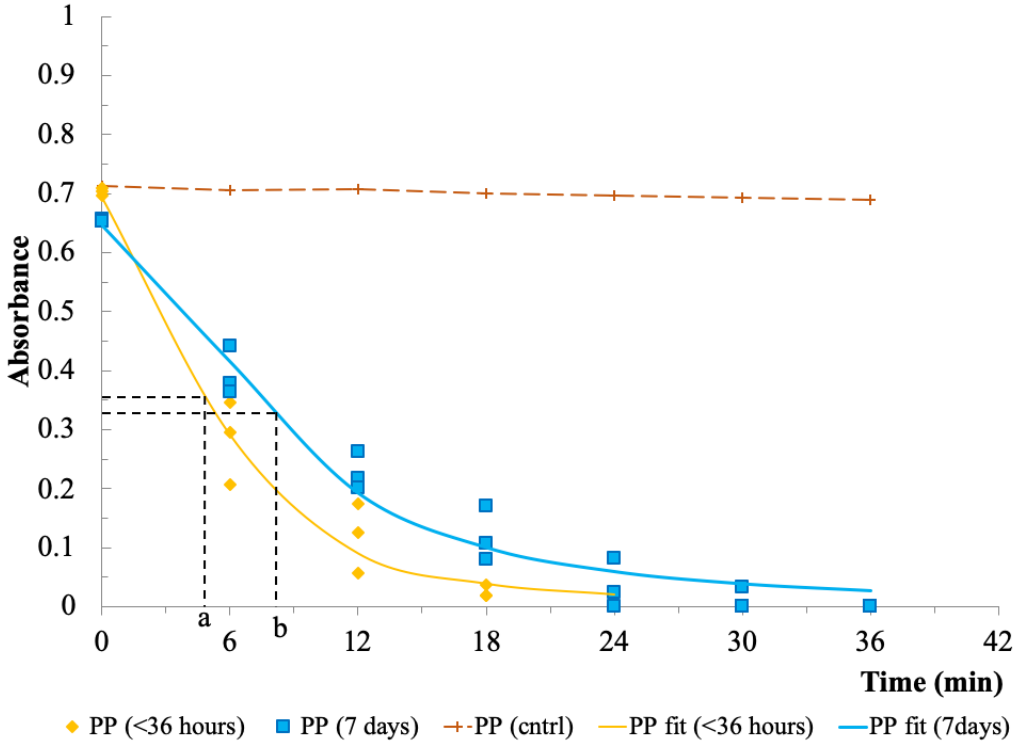
It is critical to note that the P-values obtained from the statistical analyses for the overall antioxidant performance of the coated PP (< 36 hours) and PP (7 days) were greater than 0.05 for both $ABTS^{\bullet+}$ evaluations. This indicated that the differences between the free radical reduction rates at some time intervals did not introduce statistically significant difference to the overall performance. Coated PP films with longer than 7 days of storage time should be tested to further understand which crude extract could generate a phenolic coating layer with longer effectiveness.



A. Dose-response curves

Figure 5.16 Antioxidant activity of coated PP films with different storage times. The films were coated in alkaline saline (pH = 7.8) solution with acetonic seed extract. The ABTS$\bullet^{+}$ solvent was diluted with 95% aqueous ethanol. Points a and b are the estimated ET_{50} of PP (< 36 hours) and PP (7 days) respectively.

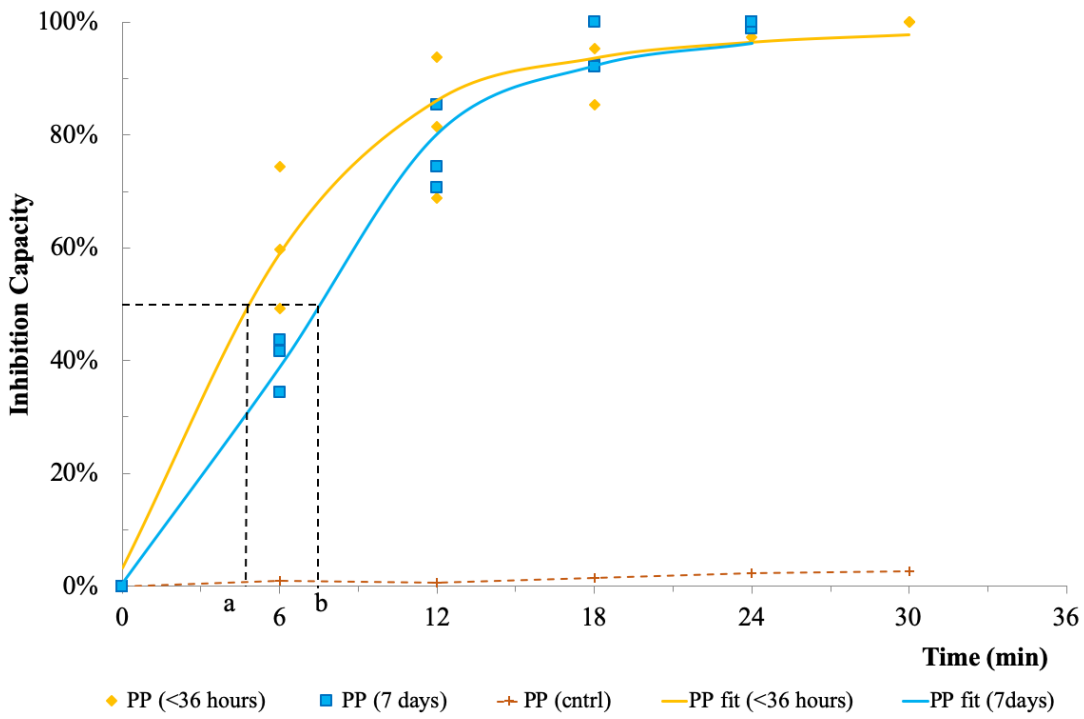
Figure 5.16 (cont'd)



B. Boltzmann sigmoidal curves

Table 5.5 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the film storage time vs. antioxidant efficacy test. ABTS•$^{+}$ assay was applied to evaluate polymer films coated with the avocado seed extract.

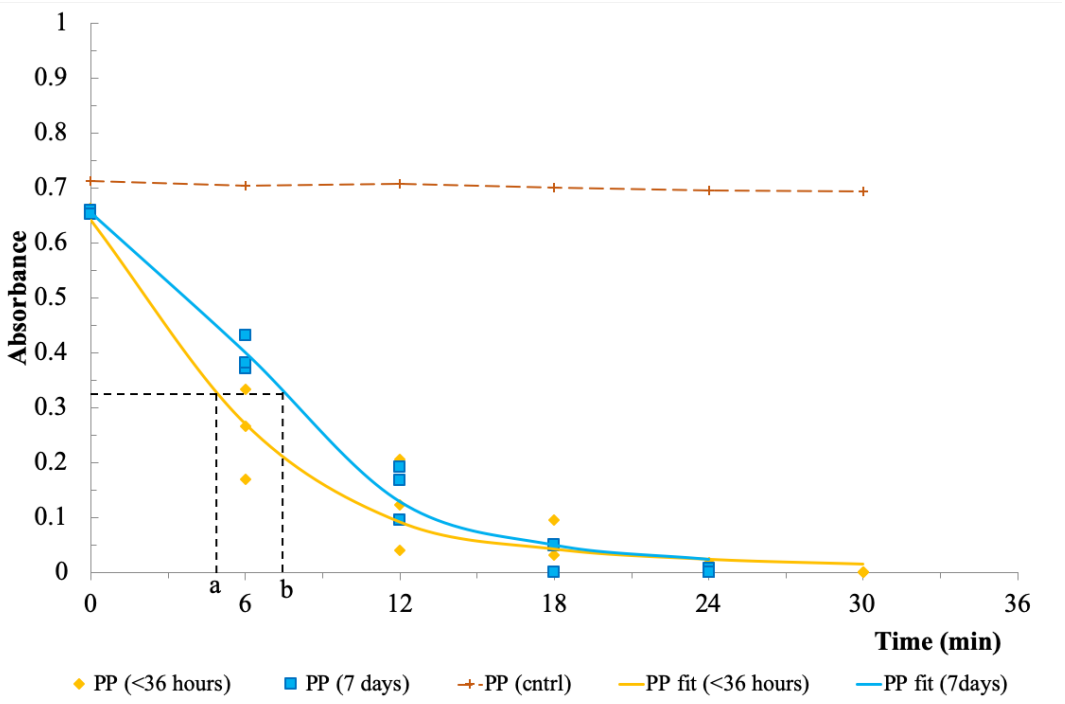
	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
PP (<36 hours)	5.17 ± 1.07	5.10 ± 1.07	0.978	0.978	0.060	0.041
PP (7 days)	7.86 ± 1.05	7.84 ± 1.05	0.975	0.975	0.057	0.037



A. Dose-response curves

Figure 5.17 Antioxidant activity of coated PP films with different storage times. The films were coated in alkaline saline (pH = 7.8) solution with acetonic peel extract. The ABTS$\bullet^{+}$ solvent was diluted with 95% aqueous ethanol. Points a and b are the estimated ET_{50} of PP (< 36 hours) and PP (7 days) respectively.

Figure 5.17 (cont'd)



B. Boltzmann sigmoidal curves

Table 5.6 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the film storage time vs. antioxidant efficacy test. $ABTS^{\bullet+}$ assay was applied to evaluate polymer films coated with the avocado peel extract.

	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
PP (<36 hours)	5.05 ± 1.10	5.03 ± 1.10	0.961	0.962	0.075	0.049
PP (7 days)	7.11 ± 1.04	7.08 ± 1.04	0.986	0.986	0.048	0.031

Table 5.7 Antioxidative efficacy of coated PP films with different storage times. The sample films were coated with acetonic peel and seed extracts in alkaline saline (pH = 7.8) solution. ABTS$\bullet^{+}$ radicals in 95% aqueous ethanol was used as the evaluation solution.

Time (min)	Inhibition Capacity % (peel extract)		Inhibition Capacity % (seed extract)	
	PP (< 36 hours)	**PP (7days)**	**PP (< 36 hours)**	**PP (7days)**
0	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
6	61.08 ± 12.65%^{a}	39.87 ± 4.91%^{b}	59.76 ± 10.28%^{a}	39.71 ± 6.31%^{b}
12	81.35 ± 12.51%^{a}	76.83 ± 7.65%^{a}	83.06 ± 8.52%^{a}	65.32 ± 4.96%^{b}
18	93.57 ± 7.46%^{a}	94.90 ± 4.42%^{a}	96.48 ± 1.56%^{a}	81.77 ± 7.00%^{b}
24	99.14 ± 1.49%^{a}	99.60 ± 0.69%^{a}	99.90 ± 0.17%^{a}	94.60 ± 6.45%^{a}
30	100.00 ± 0.00%			98.32 ± 2.91%
36				100.00 ± 0.00 %

The inhibition capacity % is presented as mean value ± standard deviation

Values with different superscripts indicate a statistically significant difference between the antioxidant effectiveness of the PP (< 36 hours) and PP (7 days) films during the same time interval

5.2.3 Extract ratio vs. antioxidant efficacy

In 2016, Calderón-Oliver et al. reported that the avocado peel extract (Hass variety) applied in their research acted as a better antioxidant than the avocado seed extract. When the avocado seed and peel extracts were blended together at different ratios, the antioxidant efficacy of those mixtures varied. In this research, significant difference between the antioxidant efficiencies of the avocado seed and peel extracts was also observed. When directly testing the free radical elimination ability of the extracts from avocado byproducts, the peel extract presented more potent efficacy than the seed extract (section 5.1.3.4.1).

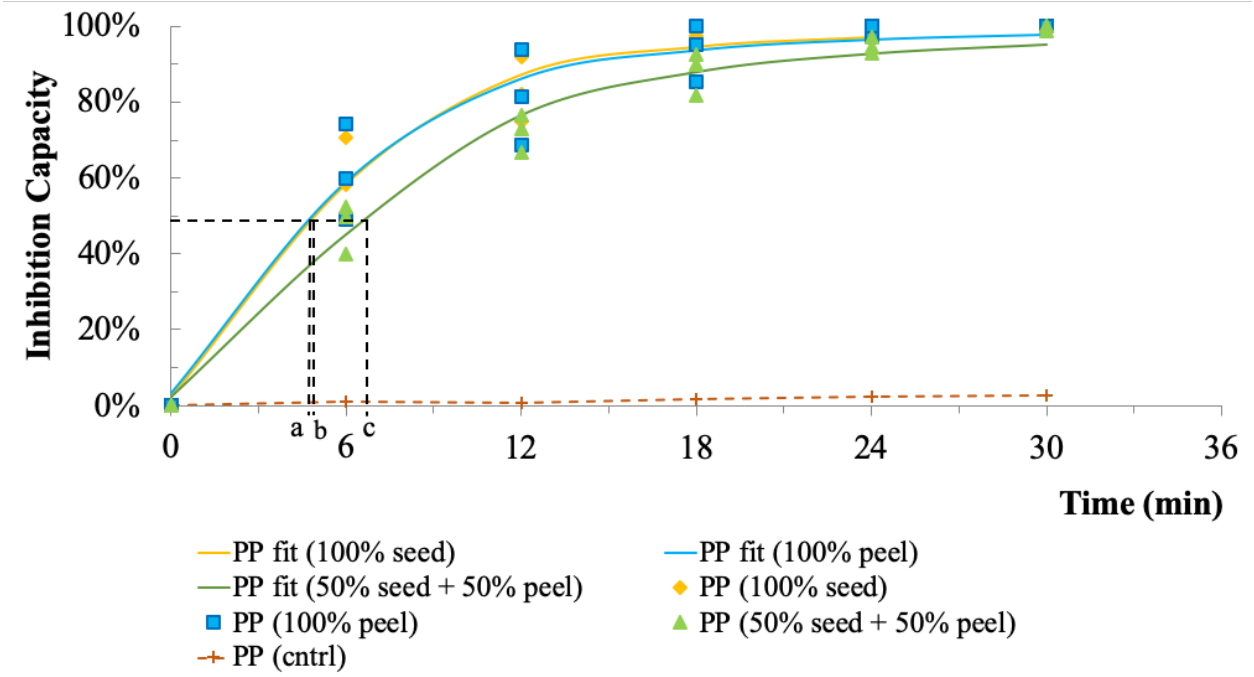
Therefore, evaluating the correlation between the phenolic extract ratio ($\frac{W_{peel\ extract}}{W_{seed\ extract}}$) in the coating solution and the antioxidant efficacy of the coating layer is necessary. PP and LDPE films were selected for this test to be coated with acetonic phenolic extracts in the alkaline saline solution. The evaluated $\frac{W_{peel\ extract}}{W_{seed\ extract}}$ ratios were 100% to 0%, 0% to 100%, and 50% to 50%. $ABTS^{\bullet+}$ stock solution diluted with 95% aqueous ethanol was used for the analysis.

As presented in Figure 5.18, the PP films coated with 100% peel extract and 100% seed extract required almost the same amount of time to reduce 50% of the $ABTS^{\bullet+}$ radicals, while the sample film coated with the mixture of the byproduct extracts reached its ET_{50} point around 1.5 minutes later (Table 5.8). When comparing the entire reaction time, no statistically significant difference was obtained among the coated PP films (Table 5.9). However, evaluations

for the antioxidant efficiency of the coated PP films at each time interval revealed significant differences among the sample films during the first 12 minutes (Table 5.10). Compared with PP (100% peel) and PP (100% seed) films, the PP (50% peel + 50% seed) films had significantly lower efficiency within the first 6 minutes. As time increased, the difference gradually decreased and there was no statistically significant difference with PP (100% peel) films at 12 minutes and with PP (100% seed) films at 18 minutes.

As shown in figure 5.19, slight differences were observed among the antioxidant efficiencies of the coated LDPE films. The substrates coated with the mixture of the byproduct extracts most rapidly reduced 50% of the $ABTS^{\bullet+}$ radicals, followed by the sample film coated with 100% peel extract. The LDPE (100% seed) films presented almost the same antioxidant process as the LDPE (100% peel) films. There was at most 0.38-minute difference between the estimated ET_{50} of the LDPE (100% peel) and LDPE (100% seed) films (Table 5.11). No statistically significant difference was found from the overall reaction time comparisons and the analyses of the antioxidant efficiency at each time interval (Table 5.12 and 5.13).

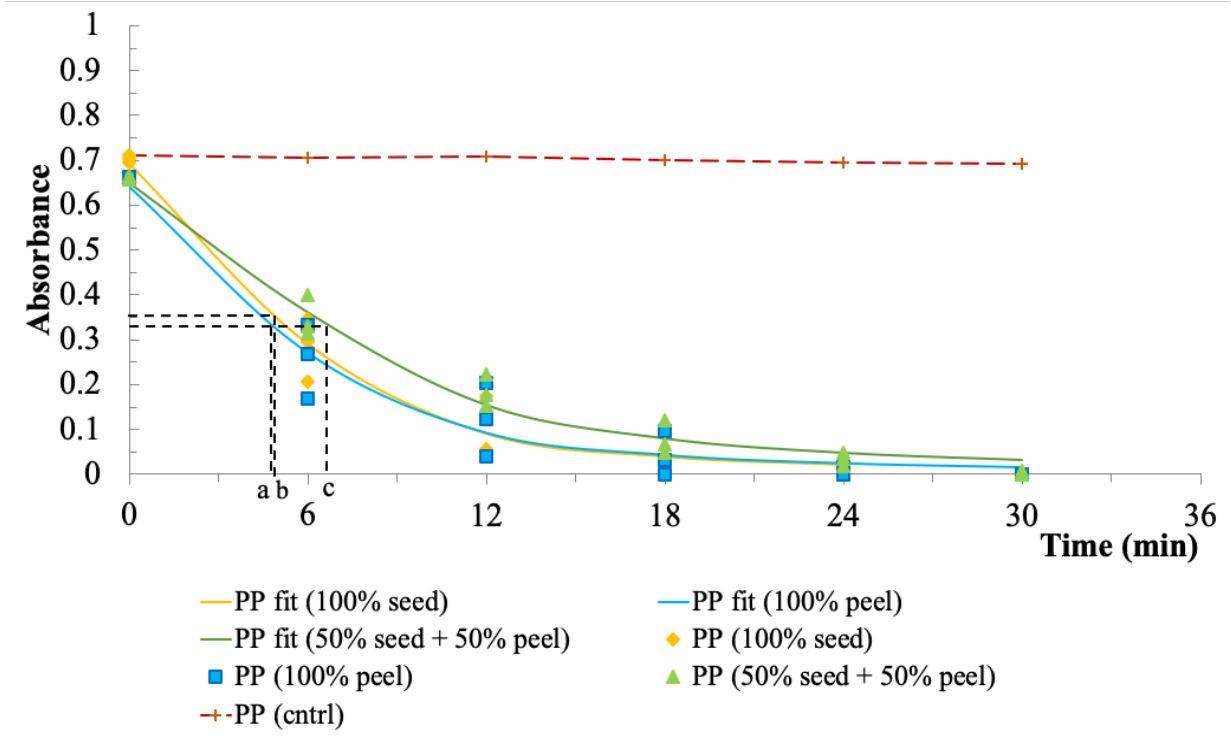
These analysis results implied that even though variances were introduced to the antioxidant efficiency of polymer films coated with the extracts at different ratios, the phenolic extract composition in the coating solution did not significantly affect the overall antioxidant activity of the coating layer.



A. Dose-response curves

Figure 5.18 Antioxidant activity of PP films coated with the phenolic extracts from avocado byproducts at different ratios. The films were coated in alkaline saline (pH = 7.8) solution. The ABTS•$^{+}$ solvent was diluted with 95% aqueous ethanol. Points a, b and c are the estimated ET_{50} of PP (100% peel), PP (100% seed) and PP (50% seed + 50% peel) respectively.

Figure 5.18 (cont'd)



B. Boltzmann sigmoidal curves

Table 5.8 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the extract ratio vs. antioxidant efficacy. ABTS•$^{+}$ assay was applied to evaluate coated PP films.

	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
PP (100% seed)	5.17 ± 1.07	5.10 ± 1.07	0.977	0.978	0.060	0.041
PP (100% peel)	5.05 ± 1.10	5.03 ± 1.10	0.961	0.962	0.075	0.049
PP (50% seed + 50% peel)	6.61 ± 1.05	6.54 ± 1.05	0.983	0.983	0.048	0.032

Table 5.9 Reaction time comparisons for PP films coated with the phenolic extracts from avocado byproducts at different ratios. ABTS•$^{+}$ assay was applied for the free radical reduction test.

	PP (100% peel)	**PP (100% seed)**	**PP (50% peel + 50% seed)**
ET_{Total} Time (min)	30 [a]	24 [a]	30 [a]

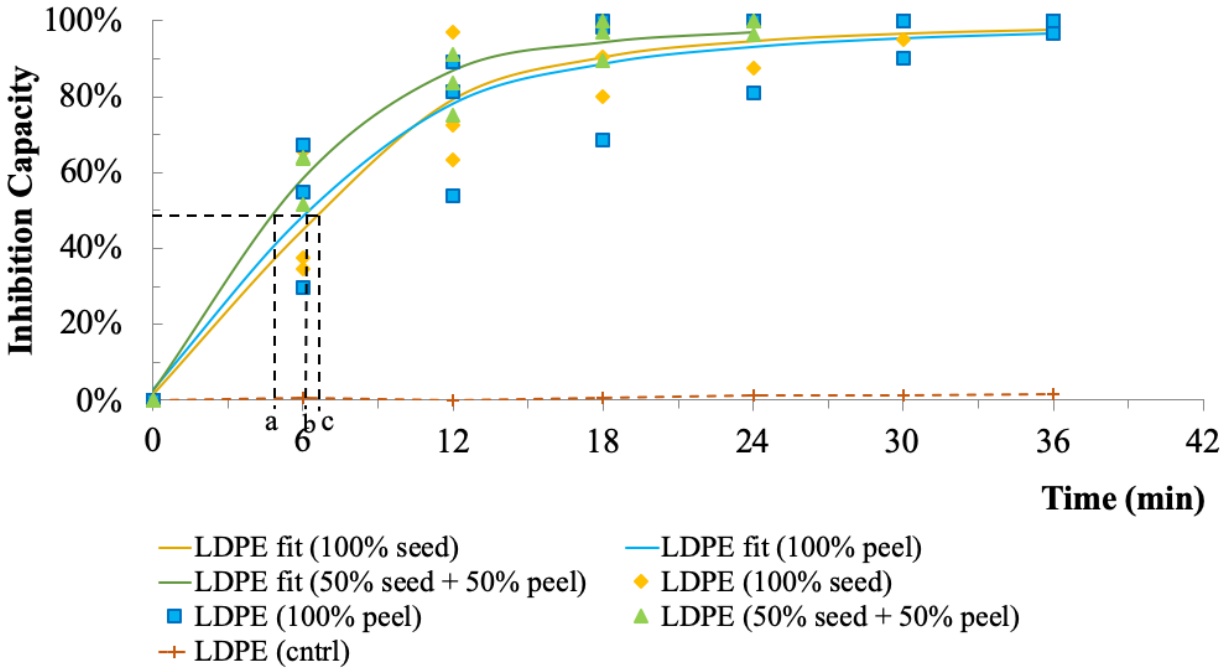
Values with the same superscript indicate no statistically significant difference among the overall reaction times used for the ABTS•$^{+}$ tests

Table 5.10 Comparisons for the antioxidative efficiency of PP films coated with the phenolic extracts from avocado byproducts at different ratios. The sample films were coated in alkaline saline (pH = 7.8) and tested in ABTS$\bullet^{+}$ solution diluted with 95% aqueous ethanol.

Time (min)	Inhibition Capacity %		
	PP (100% peel)	**PP (100% seed)**	**PP (50% peel + 50% seed)**
0	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
6	61.08 ± 12.65%[a]	59.76 ± 10.28%[a]	47.46 ± 6.51%[b]
12	81.35 ± 12.51% [a, c]	83.06 ± 8.52% [a]	72.07 ± 5.02%[b, c]
18	93.57 ± 7.46% [a]	96.48 ± 1.56%[a]	88.07 ± 5.57%[a]
24	99.14 ± 1.49%[a]	99.90 ± 0.17%[a]	94.86 ± 2.10%[a]
30	100.00 ± 0.00%[a]		99.65 ± 0.61%[a]

The inhibition capacity % is presented as mean value ± standard deviation

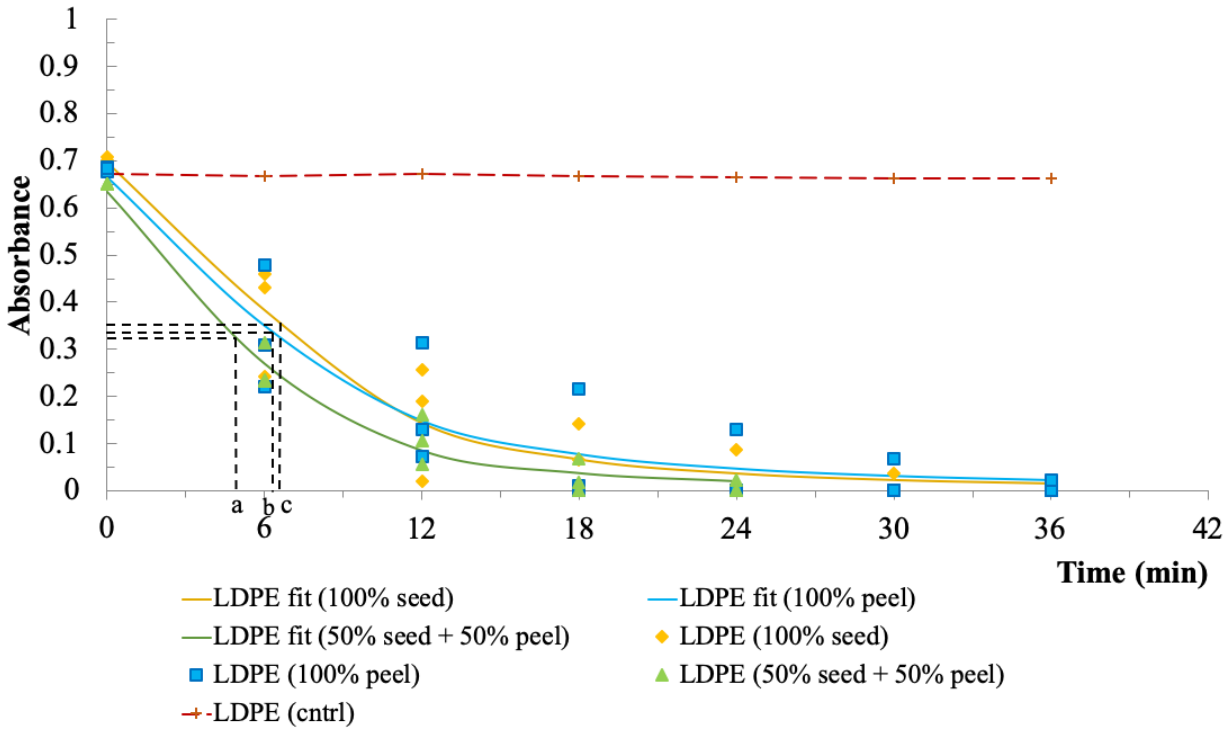
Values with different superscripts indicate a statistically significant difference among the antioxidant effectiveness of the PP films coated with the phenolic extracts at different ratios



A. Dose-response curves

Figure 5.19 Antioxidant activity of LDPE films coated with the phenolic extracts from avocado byproducts at different ratios. The films were coated in alkaline saline (pH = 7.8) solution. The ABTS•$^{+}$ solvent was diluted with 95% aqueous ethanol. Points a, b and c are the estimated ET_{50} of LDPE (50% seed + 50% peel), LDPE (100% peel) and LDPE (100% seed) respectively.

Figure 5.19 (cont'd)



B. Boltzmann sigmoidal curves

Table 5.11 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the extract ratio vs. antioxidant efficacy. ABTS•$^{+}$ assay was applied to evaluate coated LDPE films.

	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
LDPE (100% seed)	6.56 ± 1.09	6.47 ± 1.09	0.941	0.942	0.090	0.063
LDPE (100% peel)	6.18 ± 1.13	6.13 ± 1.13	0.904	0.904	0.113	0.077
LDPE (50% seed + 50% peel)	5.14 ± 1.07	5.14 ± 1.07	0.981	0.981	0.054	0.035

Table 5.12 Reaction time comparisons for LDPE films coated with the phenolic extracts from avocado byproducts at different ratios. ABTS•$^{+}$ assay was applied for the antioxidant test.

	LDPE (100% peel)	**LDPE (100% seed)**	**LDPE (50% peel + 50% seed)**
ET_{Total} Time (min)	36[a]	36[a]	24[a]

Values with the same superscript indicate no statistically significant difference among the overall reaction times used for the ABTS•$^{+}$ tests

Table 5.13 Comparisons for the antioxidative efficiency of LDPE films coated with the phenolic extracts from avocado byproducts at different ratios. The sample films were coated in alkaline saline (pH = 7.8) and tested in ABTS$\bullet^{+}$ solution diluted with 95% aqueous ethanol.

Time (min)	Inhibition Capacity %		
	LDPE (100% peel)	**LDPE (100% seed)**	**LDPE (50% peel + 50% seed)**
0	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
6	50.63 ± 19.16%[a]	45.87 ± 17.15%[a]	59.83 ± 7.13%[a]
12	74.76 ± 18.39%[a]	77.67 ± 17.34%[a]	83.33 ± 8.06%[a]
18	88.97 ± 17.73%[a]	90.10 ± 10.05%[a]	95.57 ± 5.37%[a]
24	93.66 ± 10.86%[a]	95.87 ± 7.16 [a]	98.83 ± 2.02%[a]
30	96.73 ± 5.66%[a]	98.30 ± 2.94%[a]	
36	98.88 ± 1.94%[a]	99.23 ± 1.33% [a]	

The inhibition capacity % is presented as mean value ± standard deviation

Values with the same superscript indicate no statistically significant difference among the antioxidant effectiveness of the LDPE films coated with the phenolic extracts at different ratios

To sum up, the ABTS$\bullet^{+}$ assay was employed at this stage to evaluate the potential influence of plastic substrate, storage time of coated film, and the phenolic extract ratio in the coating solution on the antioxidant efficiency of the phenolic coating layer.

Based on the dose-response and Boltzmann estimation curves and the statistical analysis, the plastic substrates did not cause any statistically significant difference in the antioxidant effectiveness of the phenolic coating. This outcome, again, agreed with the experimental results from the previous silver nitrate, DPPH$\bullet$ and ABTS$\bullet^{+}$ screening tests.

Even though no significant variance was found among the overall antioxidant rates of coated PP films with different storage times, the antioxidant efficiencies of the coated films did vary significantly during some time intervals. Therefore, coated films with longer than 7 days of storage time should be tested to further understand which phenolic extract could provide a coating layer with longer effectiveness.

Although the avocado peel extract exhibited more potent antioxidant efficiency than the seed extract, no statistically significant difference was found among the polymer substrates coated with the phenolic extracts from avocado byproducts at different ratios. On the contrary, the coated films presented similar performance in stabilizing ABTS$\bullet^{+}$ radicals in the working solution.

CHAPTER SIX:

Mode of Antioxidant Activity of Coated Polymer Films

6.1 Introduction

In this chapter, experiments were separated into two parts to evaluate the mode of antioxidant activity of the phenolic coating, i.e. antioxidative recoverability of the phenolic coating and phenolic compound releasing experiment. Based on its stability in various pH environments, solubility in aqueous and organic solutions, and short reaction time, the $ABTS^{\bullet+}$ assay (section 5.1.3) was employed to test the antioxidative recoverability of the same piece of coated film for five trials. If phenolic compounds in the coating layer could recover their antioxidant ability, the $ABTS^{\bullet+}$ radicals used for the five trials should be completely reduced. In addition, the reaction times required for the five-trial experiments should vary within an acceptable range.

After the initial and second trials of the $ABTS^{\bullet+}$ test for the coated film, the remaining food simulant, hereafter referred to as the after-reaction solution, was collected separately for a screening test. This screening test was performed to determine whether phenolic compounds were released into the working solution during the free radical elimination process. After the existence of such compounds was confirmed, a migration test was later carried out to evaluate the release process.

For both parts of the experiments, 95% aqueous ethanol was used as a food simulant (fatty food simulant) to dilute the $ABTS^{\bullet+}$ stock solution and to directly contact the coated films for the migration test.

As concluded from chapter five, the alkaline saline solution (section 4.3.2), compared with the laccase assist coating solution (section 4.3.1), could provide sample films a potent phenolic coating layer with higher antioxidant efficiency. Therefore, this coating method was utilized for this chapter to prepare coated sample films.

To obtain a thorough understanding of the antioxidant coating, ET_{50} was used again. It refers to the effective time required for the phenolic coating layer to reduce 50% of $ABTS^{\bullet+}$ radicals in the working solution. Its value was determined by fitting experimental data in the dose-response and Boltzmann sigmoidal mathematical models discussed in section 5.2.

For the statistical analysis, IBM® SPSS (SPSS Inc., Chicago, IL, USA) and JMP Pro 14 (SAS Institute Inc., Cary, NC, USA) programs were utilized. Figure C-1 and Table C-1 in Appendix C include the analysis results regarding the goodness of fit and fitted regression equations estimated by the modified Boltzmann sigmoidal (Equation 5.3) and dose-response (Equation 5.4) models used for the antioxidative recoverability test.

6.2 Antioxidative recoverability test

6.2.1 Materials

PP films (1.5 × 2 cm) were coated with the acetonic peel extract by using the alkaline saline coating method (section 4.3.2).

6.2.2 Methods

The ABTS•$^{+}$ assay elaborated in section 5.1.3 was carried out to evaluate antioxidative recoverability of the same piece of coated film in five trials. On the first day, three consecutive trials of the ABTS•$^{+}$ assay were performed. Then, the tested film was removed from the ABTS•$^{+}$ working solution and stored at room temperature. On day 7, the coated PP film used for the first three trials of the ABTS•$^{+}$ assay was tested for two more consecutive trials. For the purpose of statistical analysis, triplicates were used.

Between two consecutive trials, the tested PP film was removed from the previous working solution, rinsed with DI water, and purged by pure nitrogen flow to remove residues left on the film surface. After that, it was immersed into another ABTS•$^{+}$ working solution for the next trial.

6.2.3 Results and discussion

On the first day, the coated PP film took 16, 32, and 54 minutes respectively to completely eliminate ABTS•$^{+}$ radicals in the first three trials. As the number of testing trials

increased, the coated sample films required comparatively longer times for the free radical reduction in each working solution. Based on the LSD analysis for pairwise comparisons, there was statistically significant difference between the total time span for $ABTS^{\bullet+}$ elimination (ET_{Total}) of trials 2 and 3 (Figure 6.1).

After 5 days of recovery, the same sample films used for the first three trials on day 1 restored antioxidant ability to some extent. Under this scenario, enhanced antioxidant efficiency was observed in the last two trials on day 7. Compared with trial 3, the same coated films shortened their reaction time for ET_{Total} by 44.4% (Figure 6.1) and reduced ET_{50} by at least 12.7% in trial 4 based on the dose-response and Boltzmann sigmoidal results shown in Figure 6.2 and Table 6.1. Statistically, the ET_{Total} of trial 4 was not significantly different from the ET_{Total} of trials 1 and 2. In other words, the 5-day recovery allowed the coated PP films to regain their antioxidant efficacy so that shorter reaction time and recovered antioxidant efficiency were observed in trial 4 on day 7. In the last trial, the sample films used 48 minutes on average to completely quench $ABTS^{\bullet+}$ radicals, which was not statistically significant different from the ET_{Total} of trial 3.

As for the antioxidant efficacy, the steeper slope of trial 4 fitting curves shown in Figure 6.2, from around 6 to 20 minutes, evidenced a greater antioxidant efficiency than trials 2, 3, and 5 during the same time interval. This higher efficiency might result from the restored antioxidant

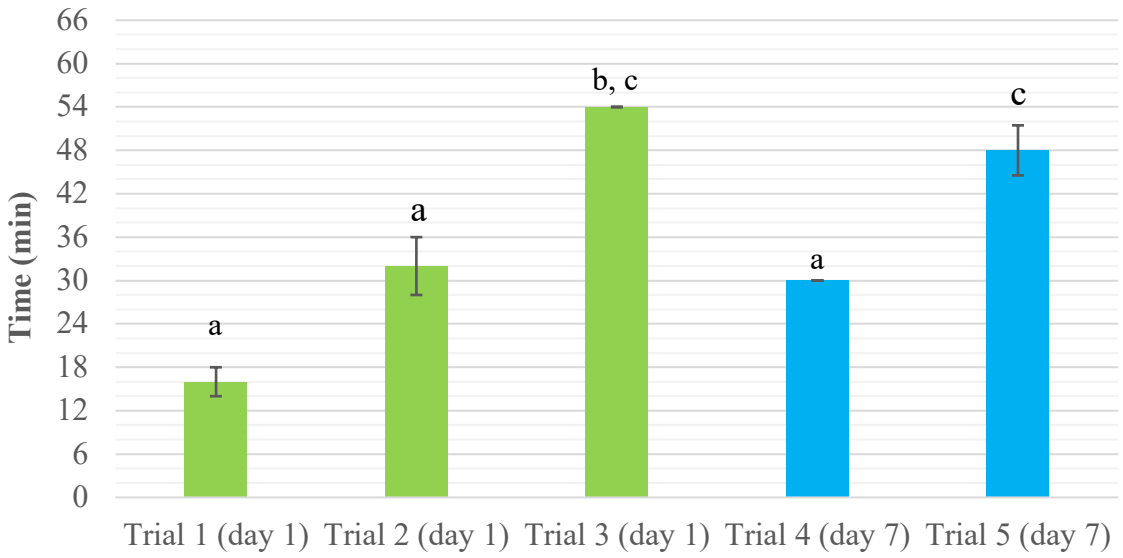
ability of the phenolic coating, and it enabled the coated sample films to use less time than trials 3 and 5 to reach the ET_{50} point (Table 6.1), and finish 100% of $ABTS^{\bullet+}$ elimination in 30 minutes. Comparing the antioxidant ability of trial 4 with trial 2 at each time interval (Table 6.2A), a statistically significant difference was noticed within the first 12 minutes. However, the greater antioxidant efficiency of trial 4 allowed the difference to be gradually decreased. Finally, no significant difference was observed between these two trials at 18 minutes.

While observing the fitting curves for trials 3 and 5, similar antioxidant performance was found. Based on the pairwise comparison result (Table 6.2B), the same sample films exhibited the same level of antioxidant efficacy at each time interval. No statistically significant difference was obtained.

If the coating layer gradually released phenolic compounds into the $ABTS^{\bullet+}$ working solution, the reaction time would increase as the number of testing trials increased. After removing coated film from the previous working solution and placing it into a new $ABTS^{\bullet+}$ working solution, less and less phenolic compounds would remain within the coating layer to react with new free radicals. However, this was not the case. The ET_{Total} of trials 4 and 5 on day 7 were shorter than the ET_{Total} of trial 3 on day 1. No statistically significant difference was found among the ET_{Total} of trials 1, 2 (day 1) and trial 4 (day 7). In addition, the ET_{50} of trial 4

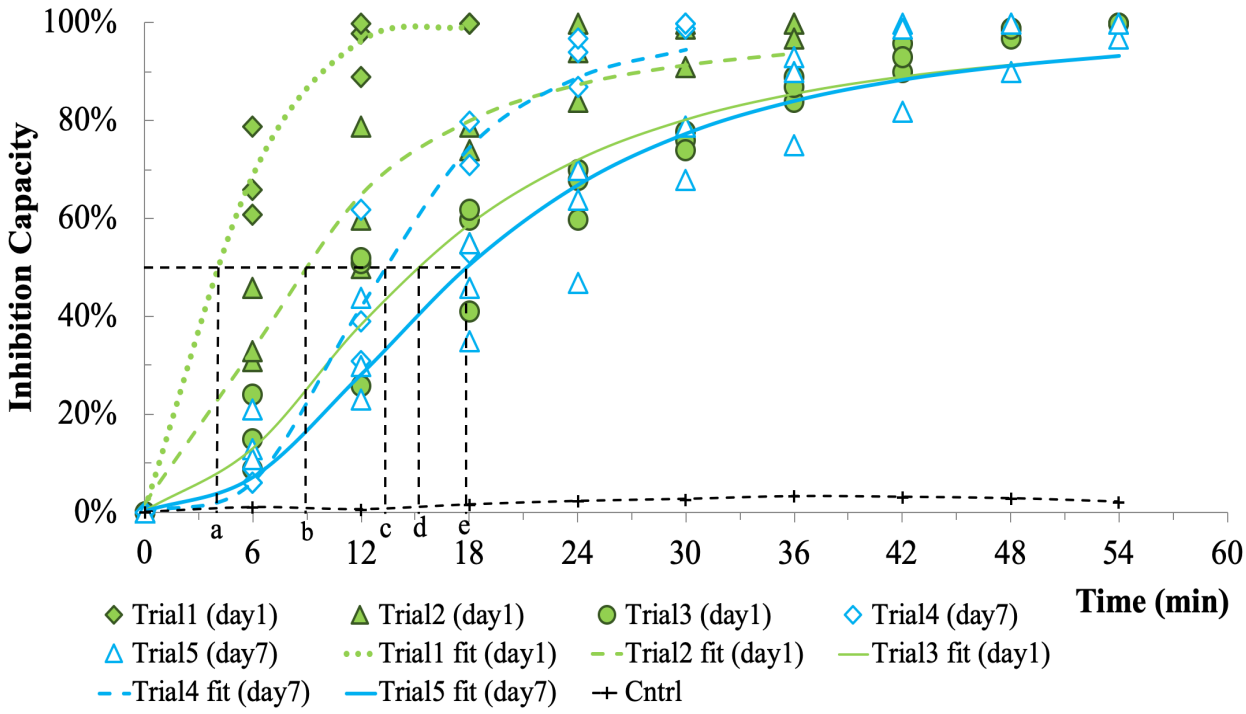
was smaller than that of trial 3. These experimental outcomes suggested that at least the majority of the phenolic compounds remained in the coating layer during those five experimental trials.

If phenolic consumption was required to eliminate $ABTS^{\bullet+}$ radicals in the five trials of working solution, the antioxidant efficacy of trials 4 and 5 would be weakened comparing with that of trials 1, 2 and 3 as the total amount of phenolic compounds that could be utilized for free radical reduction should be lessened on day 7. However, the antioxidant performance of the same coated films in trials 4 and 5 disagreed with this assumption. The films either presented improved or similar antioxidant efficiency to reduce $ABTS^{\bullet+}$ radicals compared with the first three trials. This experimental result indicated that the phenolic compounds in the antioxidant coating layer might restore their antioxidant ability to some extent during the five days of storage time.



Columns with different alphabet letters (a, b, or c) noted at the top imply that they have statistically significant difference based on the LSD pairwise comparison results for the five trials of ABTS•$^{+}$ assay.

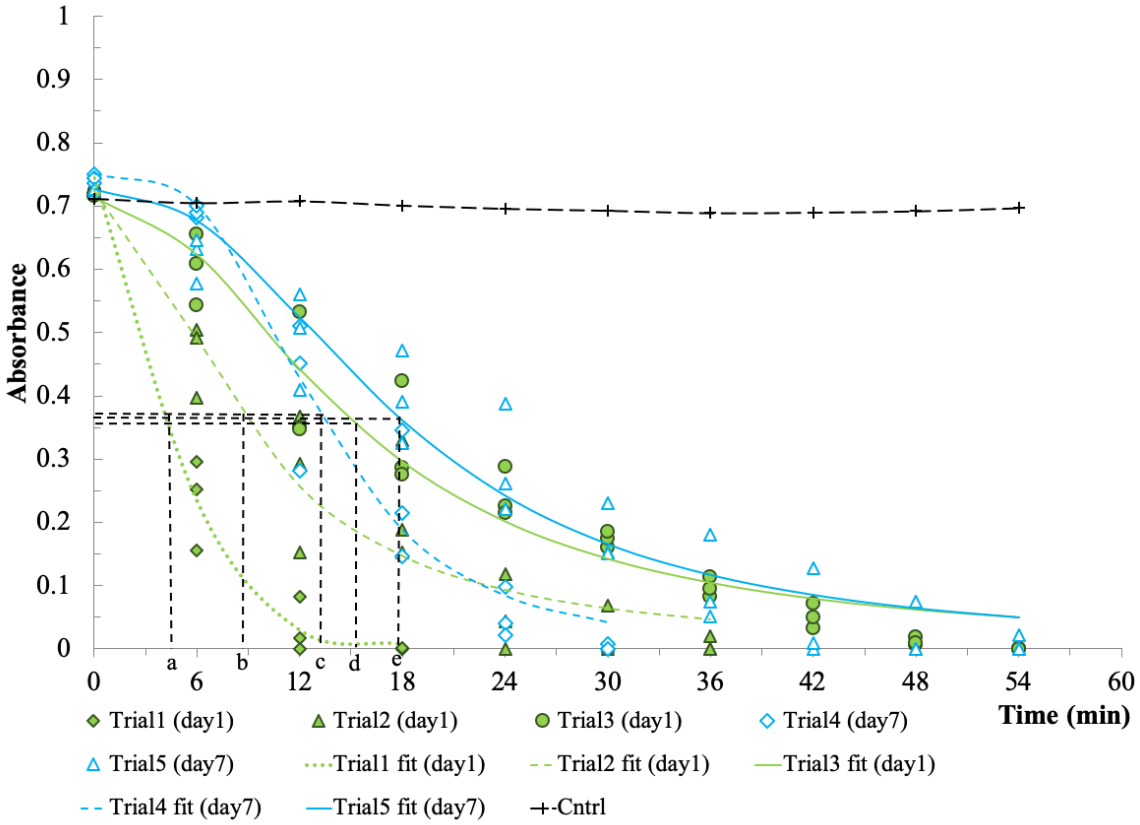
Figure 6.1 Reaction times (ET_{Total}) of the antioxidative restorability test. The alkaline saline method was applied to coat PP films with the acetonic peel extract. ABTS•$^{+}$ stock solution was diluted with 95% aqueous ethanol.



A. Dose-response curves

Figure 6.2 ABTS•$^{+}$ reduction process of the antioxidative restorability test. The alkaline saline method was applied to coat PP films with the acetonic peel extract. ABTS•$^{+}$ stock solution was diluted with 95% aqueous ethanol. Points a, b, c, d and e are the Boltzmann sigmoidal model estimated ET_{50} of trial 1, trial 2, trial 4, trial 3 and trial 5 respectively.

Figure 6.2 (cont'd)



B. Boltzmann sigmoidal curves

Table 6.1 ET_{50} values estimated by the modified dose-response and Boltzmann sigmoidal models for the antioxidative recoverability test ($ABTS^{\bullet+}$ assay).

	Estimated ET_{50} (min)		**R^2**		**RMSE**	
	Dose-response	**Boltzmann**	**Dose-response**	**Boltzmann**	**Dose-response**	**Boltzmann**
Trial 1	4.80 ± 1.08	4.78 ± 1.08	0.987	0.987	0.050	0.038
Trial 2	8.69 ± 1.09	8.64 ± 1.09	0.931	0.931	0.095	0.070
Trial 3	15.12 ± 1.05	15.14 ± 1.05	0.954	0.953	0.075	0.054
Trial 4	13.19 ± 1.05	13.04 ± 1.05	0.956	0.956	0.088	0.065
Trial 5	17.88 ± 1.05	17.88 ± 1.05	0.943	0.943	0.086	0.062

Table 6.2 Pairwise comparisons for antioxidant efficiency of the coated PP films used for the antioxidative restorability test.

Time (min)	Inhibition Capacity %	
	Trial 2 (day 1)	**Trial 4 (day 7)**
0	0.00 ± 0.00%	0.00 ± 0.00%
6	36.67 ± 8.14% [a]	7.00 ± 1.73% [b]
12	63.00 ± 14.73% [a]	44.00 ± 16.09% [b]
18	69.33 ± 12.66% [a]	68.00 ± 13.75% [a]
24	92.67 ± 8.08% [a]	92.67 ± 5.13% [a]
30	96.67 ± 4.93% [a]	99.67 ± 0.58% [a]
36	99.00 ± 1.73%	

The inhibition capacity % is presented as mean value ± standard deviation

Values with different superscripts indicate a statistically significant difference between the antioxidant efficiencies of these two trials during the same time interval

A.

Table 6.2 (cont'd)

Time (min)	Inhibition Capacity %	
	Trial 3 (day 1)	**Trial 5 (day 7)**
0	0.00 ± 0.00%	0.00 ± 0.00%
6	16.00 ± 7.55%[a]	15.00 ± 5.29% [a]
12	43.00 ± 14.73% [a]	32.33 ± 10.69% [a]
18	54.33 ± 11.59% [a]	45.33 ± 10.02% [a]
24	66.00 ± 5.29% [a]	60.33 ± 11.93% [a]
30	76.00 ± 2.00% [a]	75.33 ± 6.35% [a]
36	86.67 ± 2.52% [a]	86.00 ± 9.64% [a]
42	93.00 ± 3.00% [a]	93.67 ± 10.12% [a]
48	98.33 ± 1.15% [a]	96.67 ± 5.77% [a]
54	100.00 ± 0.00% [a]	99.00 ± 1.73% [a]

The inhibition capacity % is presented as mean value ± standard deviation

Values with the same superscript indicate no statistically significant difference between the antioxidant efficiencies of these two trails during the same time interval

B.

6.3 Phenolic compound releasing experiments

6.3.1 Screening test for released phenolic compound

6.3.1.1 Materials

The after-reaction solutions were separately collected from the initial and second trials of the previous antioxidative recoverability analysis (section 6.2).

6.3.1.2 Methods

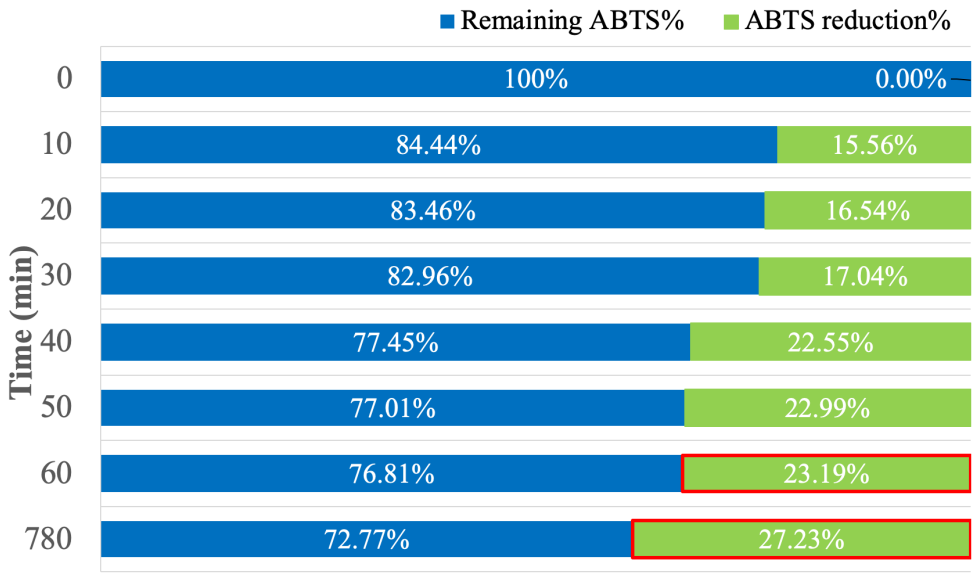
The after-reaction solution (5 μL) was added into 1 mL $ABTS^{\bullet+}$ working solution at room temperature. At each predetermined time interval, the mixture was placed in a UV-Vis spectrophotometer for absorbance measurement at 734 nm.

Light exposure was avoided during the testing process. The $ABTS^{\bullet+}$ working solution was also used as a negative control.

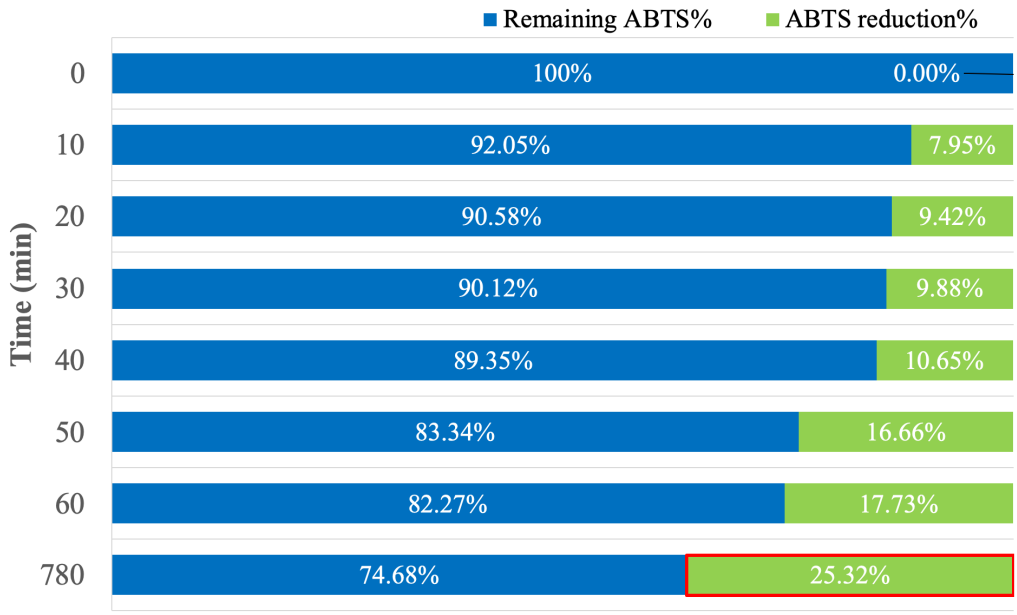
6.3.1.3 Results and Discussion

As shown in Figure 6.3A, the $ABTS^{\bullet+}$ inhibition process did occur in the mixture, implying the presence of released phenolic compounds in the after-reaction solution. However, the amount of reduced $ABTS^{\bullet+}$ radicals was relatively small and was not linear with the reaction time. It was noteworthy that at 780 minutes, $ABTS^{\bullet+}$ radicals in the negative control had been eliminated by around 4% as well (Figure 6.3C). This indicated that the antioxidant coating layer released a very limited number of phenolic compounds into the $ABTS^{\bullet+}$ working solution during

the initial trial of the antioxidant test. This small amount of released compounds could be residues left on the PP film surface after the coating process. The majority of the polymerized phenolic compounds still remained inside of the coating layer. To testify this implication, the after-reaction solution from the second trial of antioxidative restorability analysis was tested. Again, there was a very limited amount of $ABTS^{\bullet+}$ radicals eliminated from the working solution (Figure 6.3B). It is worth mentioning that both after-reaction solutions quenched almost the same amount of $ABTS^{\bullet+}$ radicals at the end of the tests. In observations of the cross-sectional SEM image of the antioxidant coating after the initial antioxidant reaction (Figure 6.4), there was no noticeable form change on the coating surface (the angled surface along the z-axis of the SEM image). The entire coating layer was still evenly distributed on the substrate.



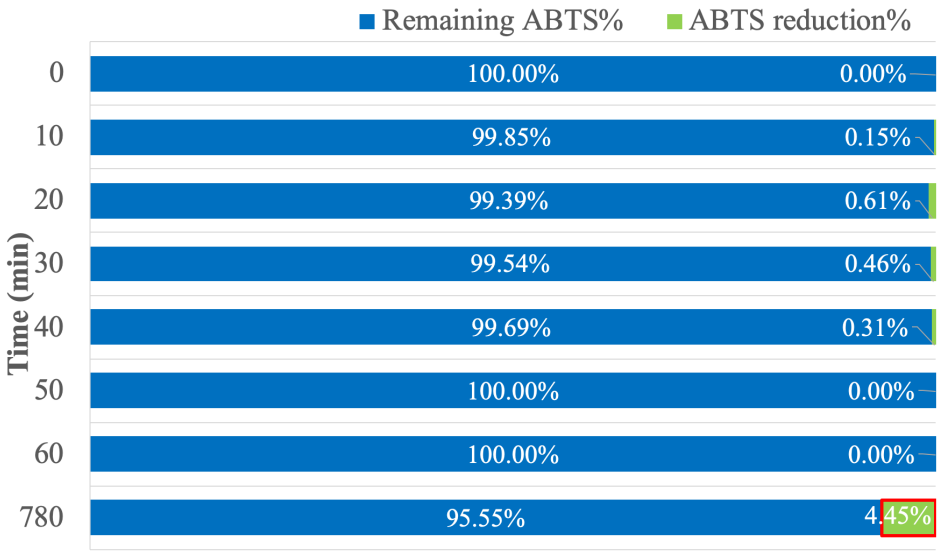
A. 5 µL after-reaction solution (after the 1st trial) added in 1 mL ABTS•+ working solution



B. 5 µL after-reaction solution (after the 2nd trial) added in 1 mL ABTS•+ working solution

Figure 6.3 Antioxidant efficiency of the after-reaction solutions collected from the antioxidative restorability analysis. ABTS•+ assay was used for the evaluation.

Figure 6.3 (cont'd)



C. Control sample (1 mL ABTS•$^{+}$ working solution)

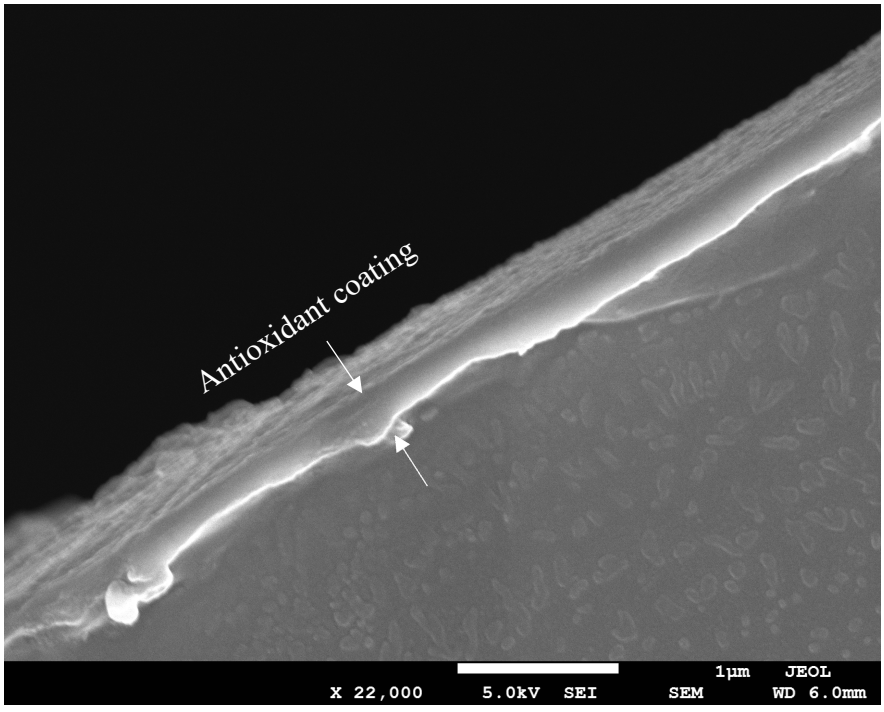


Figure 6.4 Cross-sectional SEM image of a coated PP film (alkaline saline coating method) after its initial antioxidative reaction with ABTS•$^{+}$ radicals.

6.3.2 Migration test

6.3.2.1 Materials

High performance glass vials (20 mL) were supplied by PerkinElmer, Inc. (Waltham, MA, U.S.A.). Injection syringes (1 mL) were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, U.S.A.). Stainless steel wire and glass beads (diameter: 3 mm) were obtained from a local Hobby Lobby store.

Eighteen pieces of 4.5 × 2 cm PP films were coated with the acetonic peel extract by employing the alkaline saline coating method described in section 4.3.2.

6.3.2.2 Methods

This migration test was carried out based on ASTM D4754 – 18 (Standard Test Method for Two-Sided Liquid Extraction of Plastic Materials Using FDA Migration Cell) with modifications. Before the test, the glass vials, stainless steel wire, and glass beads were washed with DI water, and rinsed with 200 proof ethanol to remove dust particles and other contaminants. After that, they were used to make migration cells as shown in Figure 6.5. For each migration cell, a 4.5 × 2 cm coated PP film was cut into 6 test samples with the same surface areas. These test samples were placed on the wire, spaced by glass beads, fully immersed in 17 mL food simulant (95% ethanol, 50% ethanol, or 10% ethanol), and kept at 4 or 40 °C for 7 days. Triplicates were used to test the coated PP films directly contacting different food

simulants at each temperature, and uncoated PP films served as negative controls to verify testing results. At each predetermined time interval, 1 mL food simulant was temporarily withdrawn from each migration cell using an injection syringe and placed into a Shimadzu UV-Vis spectrophotometer (Kyoto, Japan, model: UV-1800) to measure absorbance peaks from 200 to 400 nm, in steps of 1 nm. After the measurement, the 1 mL food simulant was refilled in each migration cell, allowing further testing.

To locate the absorbance peak of the phenolic compounds in the crude extract, the avocado peel extract was directly added into food simulants at concentrations of 0.0005%, 0.001%, 0.005%, 0.01%, 0.02%, 0.04%, 0.06%, 0.08% and 0.1% (v/v) respectively for UV-Vis measurement (200 to 400 nm). The obtained UV-Vis spectra were used to establish the calibration curves so that the concentration of released phenolic compounds from the antioxidant coating could thus be estimated.

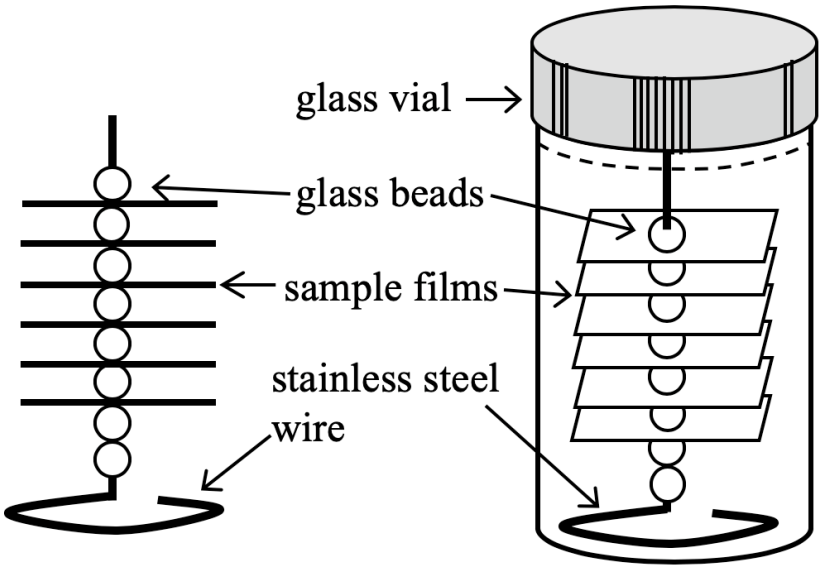


Figure 6.5 Two-sided contact migration cell for phenolic compound releasing test.

6.3.2.3 Results and discussion

Based on the UV spectra (as shown in Figure 6.6), phenolic compounds in the avocado peel extract presented an absorbance peak within the range of 280 ~ 300 nm. This absorbance peak at first exhibited negative readings indicating non-detectable phenolic compounds at low concentrations (0.0005% and 0.001%). As the concentration of the crude extract increased (> 0.001%), the absorbance peak gradually rose at around 280 nm and eventually formed a sharp peak (see Figure C-2 in Appendix C for the overlapped UV-Vis spectra of the peel extract in 50% and 10% aqueous ethanol). This peak absorbance wavelength agreed with the experimental results of other research regarding total phenolic compound detection in wine (Aleixandre-Tudo and Toit 2018; Ribereau-gayon 1974), plants (Engida et al. 2015; Owades, Rubin, and Brenner

1958), coffee and teas (Cohen 2000; Meireles et al. 2012). Although other absorbance wavelengths were also suggested, using 280 nm for phenolic compound determination is still widely accepted. For a particular phenolic compound, the absorbance wavelength may vary around 280 nm. Table 6.3 includes the suggested absorbance wavelengths for the detection of different phenolic compounds by UV-Vis spectrophotometry. These compounds are the primary antioxidants in the avocado byproduct extracts used for this research.

Considering the crude extracts used for this research were mixtures of phenolic compounds with other ingredients, the UV-Vis absorbance peak might slightly shift during the migration test. The selected wavelength range for the phenolic compound releasing experiment was 270 ~300 nm, and the concentration of released phenolic compounds from the antioxidant coating could be estimated by using the calibration curves in Figure 6.7.

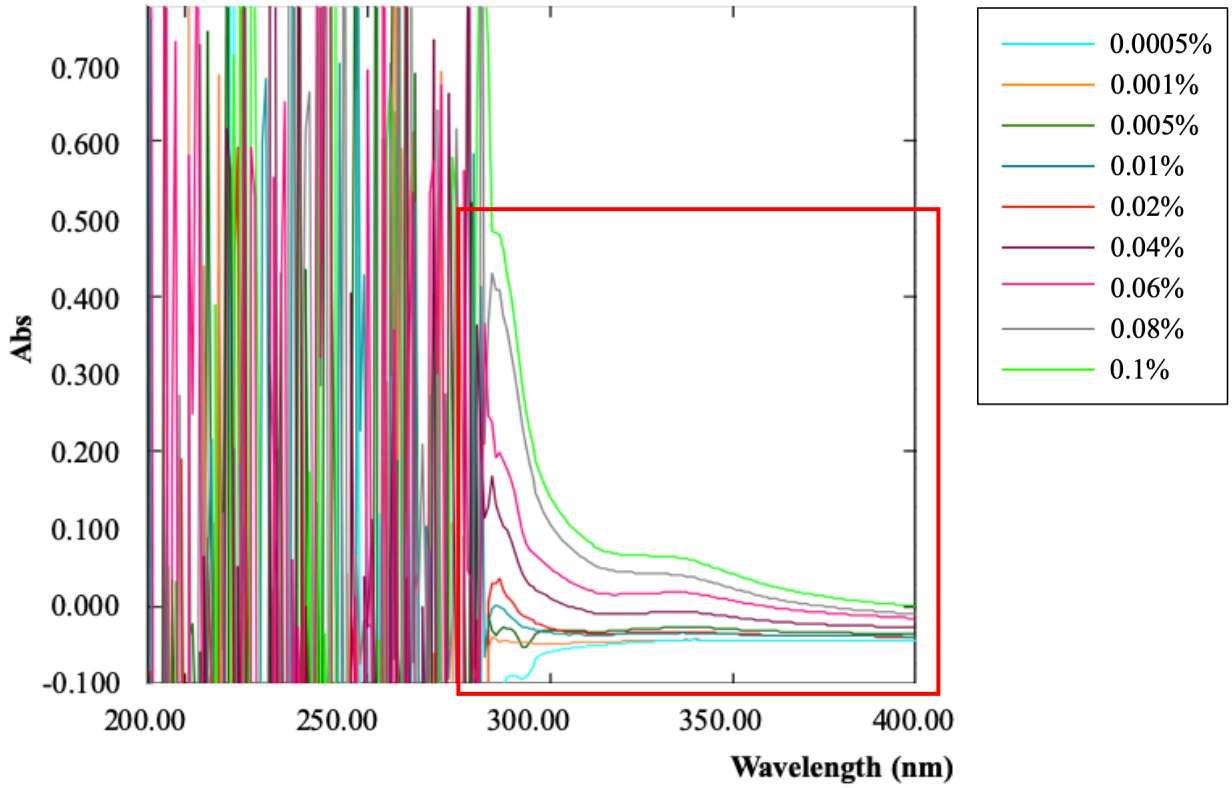
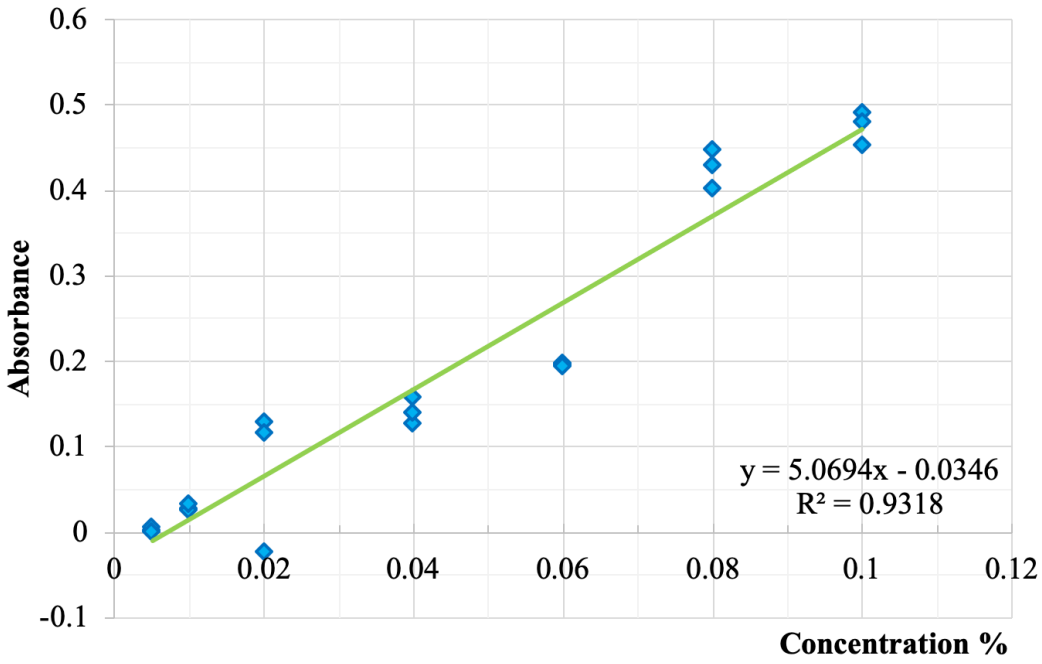


Figure 6.6 Overlapped UV-Vis spectrum of the crude phenolic extract from avocado peels in 95% aqueous ethanol at various concentrations.

Table 6.3 Absorbance wavelengths suggested for the detection of different phenolic compounds by UV-Vis spectrophotometry.

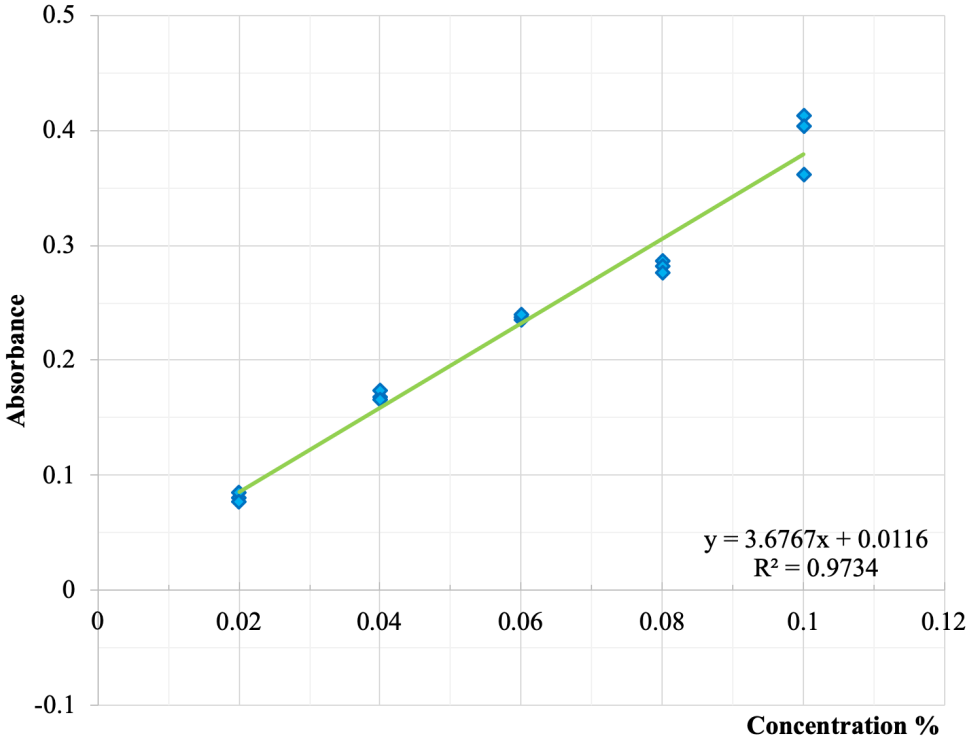
Compound Name	Absorbance Wavelength	References
Catechin	~ 288 nm	Aleixandre et al. (2018)
	275 nm	Bark et al. (2011)
5-O-caffeoylquinic acid	217 nm with shoulder at 240 nm 324 nm with shoulder at 296 nm	Belay (2010)
	~296 nm, 326 nm	Peres et al. (2013)
Procyanidin	280 nm	Wang et al. (2017)
Quercetin-3-O-rutinoside (rutin)	~ 263 nm	Aleixandre et al. (2018)
	260nm, 360 nm	Abualhasan et al. (2017)



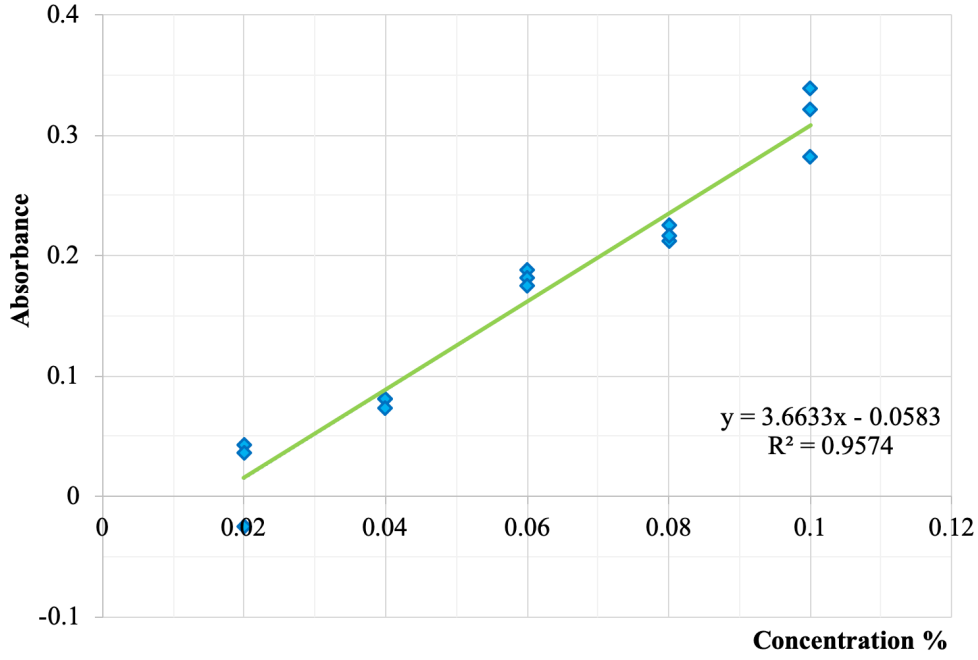
A. Avocado peel extract in 95% aqueous ethanol

Figure 6.7 Calibration curves for the phenolic compound migration analysis. The wavelength selected for the phenolic compound absorbance measurement was around 280 nm.

Figure 6.7 (cont'd)



B. Avocado peel extract in 50% aqueous ethanol



C. Avocado peel extract in 10% aqueous ethanol

Table 6.4 presents migration results of the phenolic compound releasing experiment (see Figure C-3 in Appendix C for the UV-Vis spectra of the migration results). The 'initial time' noted in the table refers to the starting time point for obtaining detectable released phenolic compounds in each migration cell. The concentration of released phenolic compounds in each migration cell was quantified using the calibration curves.

Based on the experimental results, the coated films started releasing phenolic compounds into each migration cell at different time points. Within the first 30 minutes, released phenolic compounds could be detected from 6 out of 18 migration cells. At 1 hour, the number of samples with detectable compounds increased from 6 to 10. Noticeable phenolic content was observed from two more migration cells within 24 hours. Five coated sample films did not release detectable phenolic compounds until the end of migration test.

As for the concentrations of released phenolic compounds, they did not increase as the testing time increased. Instead, they either stabilized at 0.01% during the entire testing process or ranged from 0% to 0.04%. At both experimental temperatures, the concentrations of released phenolic compounds in 10% ethanol varied within a comparatively wider range. Still, concentration stayed at a very low level. No food simulant containing more than 0.04% released phenolic compounds was found.

The aforementioned two facts verified the speculation of the previous screening test. During those 168 hours, only a small number of phenolic compounds were released from the antioxidant coating layer. It is highly possible that this limited number of compounds resulted from surface residues left by the coating process. That being said, the phenolic coating layer did not rely on a surface releasing or migration process to stop or slow down free radical reactions.

For the sample films in the same food simulants, different experimental temperatures did not have any significant influence on the release of phenolic compounds. As shown in Table 6.4, estimated concentrations of the migration cells at 4 °C were either the same or similar to that of the migration cells with the same food simulant at 40 °C. This experimental result is further evidence that the phenolic compounds from the avocado byproducts were stabilized on the substrate. They did not depend on a migration or surface releasing process for the free radical quenching process.

Since a) at least the majority of the polymerized phenolic compounds remained inside of the coating layer during the free radical reduction process, b) the coating layer could restore its antioxidant ability after quenching the $ABTS^{\bullet+}$ radicals in the working solution, and c) the diameters of the primary phenolic compounds identified from the avocado byproduct extracts, such as catechin, protocatechuic acid and rutin, were around 1 nm (see Table C-3 in Appendix C), which were much smaller than the estimated coating thickness (37.75 ± 0.30 nm, see section

4.4), the antioxidant coating layer can be presumed to have more than one layer of phenolic compounds polymerized on the polymer substrates. After donating hydrogen atoms to stabilize DPPH• or ABTS•$^{+}$ radicals, phenolic compounds at the surface layer of the antioxidant coating become phenolic radicals (Ar : O$^{\bullet}$, see Figure 6.8). In order to continuously serve as antioxidants, the surface phenolic radicals may abstract hydrogen atoms from their adjacent phenolic compounds (Ar' : OH) in an inner layer of the antioxidant coating to restore their antioxidant ability. The longer the elapsed time between two consecutive DPPH• or ABTS•$^{+}$ stabilization reactions, the more Ar : O$^{\bullet}$ at the coating surface could regain their antioxidant ability, and Ar' : OH, in this case, turn to new phenolic radicals, Ar' : O$^{\bullet}$. It is worth noting that not all the surface phenolic radicals are able to regain their antioxidant ability. Also, the O-H bond dissociation enthalpy of Ar' : OH may influence this hydrogen abstraction reaction. The lower the O-H bond dissociation enthalpy in the Ar' : OH, the more readily the hydrogen abstraction reaction will take place.

To continue the hydrogen abstraction reaction between Ar : O$^{\bullet}$ and Ar' : OH every time after the DPPH• or ABTS•$^{+}$ stabilization reaction, Ar' : O$^{\bullet}$ needs to abstract hydrogen atoms from their adjacent phenolic compounds, Ar'' : OH, to transfer the hydrogen atoms to Ar : O$^{\bullet}$ at the surface layer. As the number of the DPPH• or ABTS•$^{+}$ stabilization reaction increases, the hydrogen abstraction reaction will take place in a much deeper layer of the antioxidant coating to

transfer hydrogens, causing longer time for the surface phenolic radical, Ar : O•, to regain their antioxidant ability. Since hydrogen atoms in the phenolic coating layer would be gradually consumed, there would be less and less Ar : O• that could recover their antioxidant ability. Eventually, the phenolic coating layer will lose its antioxidant property when the last hydrogen atom is used up for the surface phenolic radicals to regain antioxidant ability.

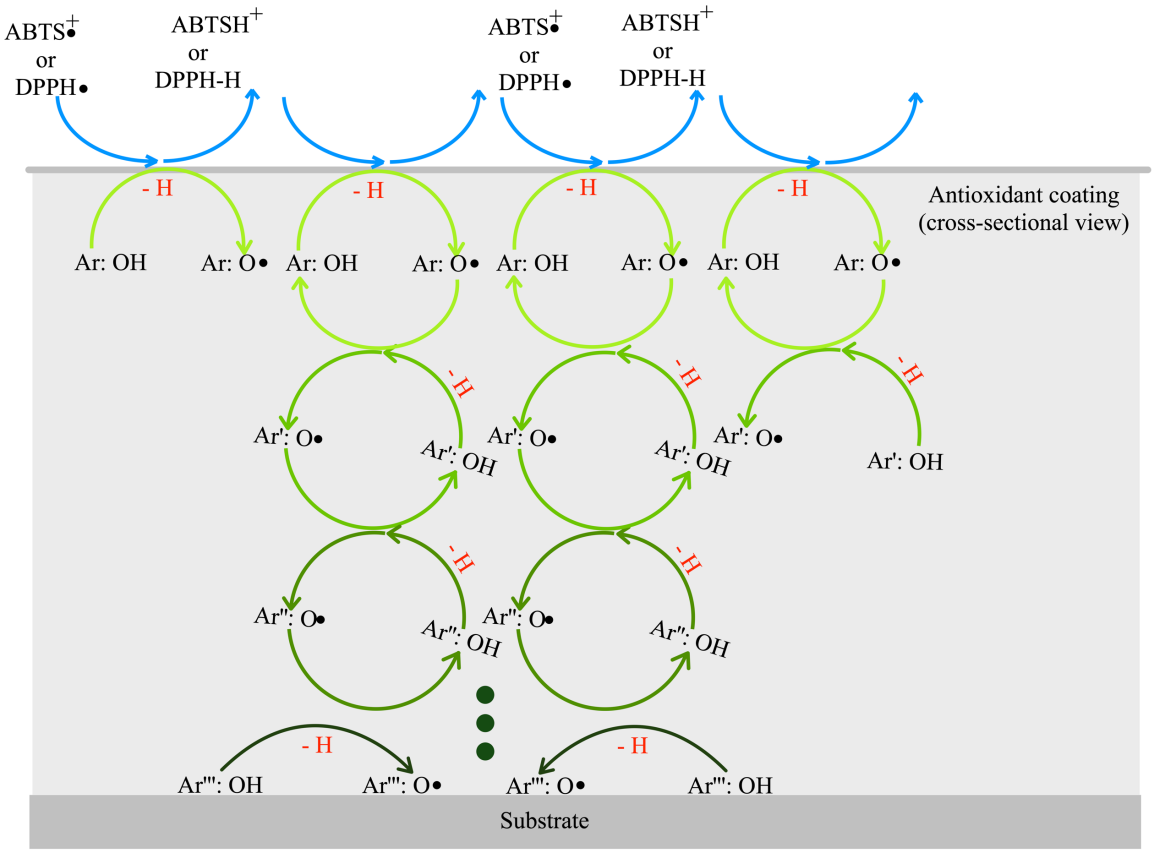
Table 6.4 Estimated concentrations of phenolic compounds released from the antioxidant coating layer during the migration test.

		95% ethanol			50% ethanol			10% ethanol		
		Cell 1	**Cell 2**	**Cell 3**	**Cell 1**	**Cell 2**	**Cell 3**	**Cell 1**	**Cell 2**	**Cell 3**
4 ◦C	**Initial Time (hours)**	0.5	0.5	1	168	168	168	24	0.5	1
	Concentration (%)	0.02 ~ 0.04	0.01 [S]	0.01 ~ 0.02	ND	ND	ND	0 ~0.04	0.02~0.04	0.02~0.04
40 ◦C	**Initial Time (hours)**	0.5	1	0.5	168	0.5	168	1	48	24
	Concentration (%)	0 ~ 0.01	0.02 ~ 0.04	0.01 ~ 0.02	ND	0 ~ 0.02	ND	0.02 ~ 0.04	0.02 ~ 0.04	0.02 ~ 0.04

Initial time refers the starting time point for obtaining detectable released phenolic compounds

The superscript S refers to the concentration of released phenolic compounds stabilized at a particular value during the entire migration test

ND refers to a non-detectable concentration of released phenolic compounds during the entire migration test



Ar : OH is a phenolic compound inside the antioxidant coating

Ar’ : OH is a phenolic compound close to Ar : OH inside the antioxidant coating

Ar’’ : OH is a phenolic compound close to Ar’ : OH inside the antioxidant coating

Ar’’’ : OH is a phenolic compound close to the plastic substrate

Ar : O• is a phenolic radical of Ar : OH

Ar’ : O• is a phenolic radical of Ar’ : OH

Ar’’ : O• is a phenolic radical of Ar’’ : OH

Ar’’’ : O• is a phenolic radical of Ar’’’ : OH

Figure 6.8 Schematic of antioxidative restorability of the phenolic coating on a plastic substrate.

CHAPTER SEVEN:

Conclusions and Future Work

7.1 Crude extraction from avocado byproducts

Both avocado peel and seed powders were utilized for this research to obtain crude phenolic extracts.

In order to maximize phenolic content recovered from the byproduct powders, 70% ethanol and 70% acetone, as the most common solvents, were selected. The selections were based on acceptability for human consumption, solvent polarity and the solubility of the extracted phenolic compounds. In addition, moderate heat treatment was used to further promote the extraction process. Within a limited time period, a Vortex mixer and ultrasonic bath, as unconventional crude extraction procedures, were utilized to allow the solvents to completely mix with avocado byproduct powders and thus increase the concentration of extracted phenolic compounds in the aqueous solvents.

7.2 Non-metal contact coating process

The coating process was a polymerization reaction. It required the involvement of oxygen and moderate mechanical agitation. More importantly, a metal contact coating technique, conventional roller coating process for example, could not be employed due to the metal

chelating ability of phenolic compounds. Considering no coating equipment was available in the lab to meet the aforementioned requirements, a non-metal contact dip coater was developed.

The dip coater applied a slider-crank mechanism to convert rotary motion into two ways of linear motion, i.e., horizontal and vertical movements. Together with a hot plate stirrer, this coating device could be used for beakers with different sizes, and the temperature of coating solution, dip coating and agitation speed were all controllable variables.

Two types of coating solutions were utilized for this research, namely, laccase assist (pH = 5) and alkaline saline (pH = 7.8) coating solutions. By mixing with the phenolic extracts at different ratios (100% peel extract, 100% seed extract, or 50% peel extract and 50% seed extract), the coating solutions successfully polymerized the phenolic compounds onto PP, LDPE, and PET films, and SEM showed the coating layer was uniform on the substrates with thickness of approximately 37.75 ± 0.30 nm.

It is worth mentioning that for the thickness measurement of the phenolic coating, a glass coverslip was used as the coating substrate. This material was able to present a distinct edge line between itself and the coating layer under SEM to facilitate the measurement. More importantly, even on a different type of substrate, the phenolic coating was still evenly distributed. This experimental result indicated the substrate-independent characteristic of the phenolic coating,

and thus implied a broader range of applications of this antioxidant coating on other types of packaging materials.

7.3 Evaluation of antioxidant efficacy of coated polymer films

To understand the antioxidant efficacy of the phenolic coating, experimental analysis was separated into two parts. In the screening test, $AgNO_3$, DPPH•, and $ABTS^{\bullet+}$ assays were employed to analyze coated PP, LDPE and PET films in food simulants based on 3 reaction mechanisms. Both avocado peel and seed extracts was used to coat the polymer films using the laccase assist and alkaline saline approaches stated in chapter four. In addition, the avocado peel and seed extracts were directly tested to understand the greater antioxidant efficiency of the peel extract.

The $AgNO_3$ method first provided a visual inspection result to prove the existence of phenolic content in the polymerized coating layer. After immersing coated polymer films into $AgNO_3$ solution, phenolic compounds in the antioxidant coating layer resulted in film color change, synthesis and deposition of silver nanoparticles. This simple testing method is recommended to preliminarily verify phenolic coating. Moreover, the darker color change on the coated polymer films indicated the higher coating efficacy of the alkaline saline method.

DPPH• radicals are purple in color. To stabilize these radicals, phenolic compounds donated single electrons and/or hydrogen atoms to gradually transform the free radical solution into a yellow color.

$ABTS^{\bullet+}$ radicals are blue-green in color. To stabilize these radicals, phenolic compounds transferred their hydrogen atoms and thus gradually removed the color in the working solution.

Based on the experimental results of DPPH• and $ABTS^{\bullet+}$ assays, the alkaline saline coating method, as an inexpensive approach, could generate a phenolic coating layer with greater antioxidant effectiveness. The polymer films coated by this approach required less time to reach ET_{50} in both DPPH• and $ABTS^{\bullet+}$ assays. Also, the different plastic substrates did not result in any statistically significant difference in terms of the antioxidant efficiency of the phenolic coating. During the coating process, the phenolic compounds acted as a layer of adhesive attached onto the plastic surface. They did not interact with the substrates to cause any variance in the antioxidant ability of the phenolic coating.

When the polymer films were coated in solutions with the same type of crude extract but different concentrations, they did not present significant difference in the antioxidant ability to reduce DPPH• radicals. It seemed that the antioxidant efficiency of the coating layer was not linearly correlated to the concentration of phenolic extract in the coating solution. Provided the coating duration and phenolic content in the coating solution were sufficient, the antioxidant

efficiency of the coating layer might be determined by substrate surface area rather than the amount of phenolic content in the coating solution.

To further evaluate the antioxidant coating, coated PP, PET and LDPE films were tested in triplicate in the second part of the experimental analysis. The statistical analyses, again, agreed that the different plastic substrates did not result in any statistically significant difference in terms of the antioxidant efficiency of the phenolic coating.

Even though no significant variance was found among the overall antioxidant rates of coated films with different storage times, the antioxidant efficiencies of the coated films did vary significantly during some time intervals.

Although the avocado peel extract exhibited more potent antioxidant efficiency than the seed extract, no statistically significant difference was found among the polymer substrates coated with the phenolic extracts at different ratios ($\frac{W_{peel\ extract}}{W_{seed\ extract}}$ = 100% to 0%, 0% to 100%, and 50% to 50% respectively). On the contrary, the coated films presented similar performance in stabilizing ABTS$^{\bullet+}$ radicals in the working solution.

7.4 Mode of antioxidant activity of coated polymer films

While stabilizing free radicals in food simulants, at least the majority of the phenolic compounds remained in the antioxidant coating layer. They did not depend on a migration or surface releasing process to quench free radicals.

According to the phenolic releasing experiments, only a small number of phenolic compounds were released into the food simulant. The majority of phenolic compounds remained in the coating layer. Possibly, this small number of migrating compounds was surface residues left by the coating process. The cross-sectional SEM image of the antioxidant coating after the initial antioxidant reaction supported this speculation. Under SEM, the phenolic coating still presented a uniform layer on the substrate; no form change was noticed.

It was also worth mentioning that the different migration test temperatures did not impact the stability of the coating layer. At 4 and 40 °C, the phenolic concentrations in the migration cells containing the same type of food simulants did not present any significant differences. This experimental result further suggested that the phenolic compounds from avocado byproducts were stabilized on the substrate. They did not depend on a migration or surface release process for free radical elimination.

Presumably, there was more than one layer of phenolic compounds extracted from the avocado byproducts polymerized on the polymer substrates. After donating hydrogen atoms to stabilize free radicals, phenolic compounds at the surface layer of the antioxidant coating became phenolic radicals. In order to continuously serve as antioxidants, the surface phenolic radicals might abstract hydrogen atoms from their adjacent phenolic compounds in an inner layer of the antioxidant coating to restore their antioxidant ability. As the number of free radical stabilization

reaction increased, the hydrogen abstraction reaction would take place in a much deeper layer of the antioxidant coating to transfer hydrogens, causing longer time for the surface phenolic radical to regain their antioxidant ability. Eventually, the phenolic coating layer would lose its antioxidant property when the last hydrogen atom was used up for the surface phenolic radicals to regain antioxidant ability.

7.5 Future work

For this research, experiments were conducted to obtain crude phenolic extracts from avocado byproducts, polymerize the crude extracts on substrates uniformly, evaluate antioxidant efficiency of the coating layer, and understand the mode of antioxidant activity of the coating layer. Still, improvements and further evaluations are required to thoroughly understand this bio-based antioxidant coating.

To obtain the antioxidant coating layer, polymer films were dip coated for 24 hours at room temperature. Although this coating duration was shorter than the experimental time recommended by other researchers, a relatively higher temperature together with a different dipping speed may expedite the coating process without introducing decomposition of phenolic compounds in the coating solution.

During each coating process, the dip coater developed for this research could only coat substrates with small surface areas. For the purpose of commercial application, a better coating

method or device, which can coat a substrate with large surface area, is desired to enhance the coating efficiency.

While measuring the coating thickness under SEM, slight deformation on the coating surface was noticed at high magnification (35000x). This deformation might result from the sample preparation process for the SEM images. After removal from liquid nitrogen, the coated substrate became very brittle. Manually fracturing the sample, in this case, might result in deformation at some spots of the coating layer. A better sample preparation method is needed to avoid any possible deformation.

Before applying the antioxidant coating in real applications, knowing the expiration time of the antioxidant coating is a must. Despite the fact that no statistically significant difference was noticed between the antioxidant efficacy of coated polymer films with less than 36 hours and 7 days of storage times, a coated film with longer storage time needs to be tested to further understand the antioxidant efficacy of the phenolic coating layer.

To better understand the antioxidant efficacy of the coating layer in real applications, food products, such as meat patties, beverage and dairy products, should be used. Different from food simulants, chemical reactions are more complicated in real food products because of their complex ingredients. If the polymerized coating layer could present its potent antioxidant efficacy to food products, it would raise its promising value in commercial use.

APPENDICES

Appendix A Antioxidant efficiency of coated polymer films (screening test results)

Table A-1 Experimental data of the coating solution vs. antioxidant efficacy test (DPPH• assay).

Time (hour)	Inhibition Capacity								
	50% aqueous ethanol			95% aqueous ethanol			50% aqueous ethanol		
	LDPE (laccase)	LDPE (alkaline)	Negative Control	LDPE (laccase)	LDPE (alkaline)	Negative Control	PET (laccase)	PET (alkaline)	Negative Control
0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
0.5	12.66%	N/A	0.00%	3.74%	56.31%	1.55%	15.98%	N/A	0.06%
1	19.78%	85.10%	0.34%	7.32%	70.87%	2.77%	32.22%	82.50%	0.12%
1.5	28.86%	85.15%	0.17%	8.93%	76.08%	4.02%	37.45%	N/A	0.17%
2	35.73%	84.62%	0.17%	9.44%	79.29%	4.26%	44.94%	80.52%	0.23%
2.5	41.76%	86.37%	0.34%	10.27%	81.77%	4.76%	50.80%	N/A	0.30%
3	47.07%	85.96%	0.07%	11.12%	83.80%	4.86%	49.67%	81.48%	0.35%
3.5	N/A	N/A	N/A	N/A	N/A	N/A	47.21%	N/A	0.48%
4	56.28%	88.56%	0.07%	17.54%	85.81%	5.12%	55.03%	82.02%	0.58%
4.5	N/A	N/A	N/A	N/A	N/A	N/A	60.97%	N/A	0.61%
5	64.15%	89.98%	1.16%	20.35%	86.65%	5.25%	66.65%	82.66%	0.74%
6	72.53%	90.28%	2.51%	21.60%	87.13%	5.29%	71.41%	N/A	1.05%
7	75.96%	90.46%	2.90%	24.18%	86.94%	5.48%	71.41%	N/A	2.34%
8	82.96%	N/A	3.55%	27.27%	86.23%	5.56%	N/A	N/A	N/A
9	86.43%	N/A	4.43%	29.67%	86.29%	6.79%	N/A	N/A	N/A
24	N/A	N/A	N/A	42.71%	86.17%	7.34%	N/A	N/A	N/A
48	N/A	N/A	N/A	63.72%	N/A	8.57%	N/A	N/A	N/A

Table A-2 Experimental data of the plastic substrates vs. antioxidant efficacy test (DPPH•

assay).

Time (hour)	Inhibition Capacity							
	50% aqueous ethanol							
	LDPE (laccase)	LDPE (alkaline)	Negative Control	PET (laccase)	PET (alkaline)	Negative Control	PP (alkaline)	Negative Control
0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
0.5	12.66%	N/A	0.00%	15.98%	N/A	0.06%	N/A	N/A
1	19.78%	85.10%	0.34%	32.22%	82.50%	0.12%	84.73%	0.35%
1.5	28.86%	N/A	0.17%	37.45%	N/A	0.17%	N/A	N/A
2	35.73%	84.60%	0.17%	44.94%	80.52%	0.23%	86.53%	2.97%
2.5	41.76%	N/A	0.34%	50.80%	N/A	0.30%	N/A	N/A
3	47.07%	86.00%	0.07%	49.67%	81.48%	0.35%	86.40%	3.01%
3.5	N/A	N/A	N/A	47.21%	N/A	0.48%	N/A	N/A
4	56.28%	88.60%	0.07%	55.03%	82.02%	0.58%	86.20%	3.25%
4.5	N/A	N/A	N/A	60.97%	N/A	0.61%	N/A	N/A
5	64.15%	90.00%	1.16%	66.65%	82.66%	0.74%	85.17%	4.11%
6	72.53%	N/A	2.51%	71.41%	N/A	1.05%	N/A	N/A
7	75.96%	N/A	2.90%	71.41%	N/A	2.34%	N/A	N/A
8	82.96%	N/A	3.55%	N/A	N/A	N/A	N/A	N/A
9	86.43%	N/A	4.43%	N/A	N/A	N/A	N/A	N/A
24	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
48	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table A-3 Experimental data of the concentration of phenolic extract vs. antioxidant efficacy test (DPPH• assay).

Time (hour)	Inhibition Capacity		
	50% aqueous ethanol		
	PP (12.5 mg/mL)	PP (25 mg/mL)	Negative Control
0	0.00%	0.00%	0.00%
0.5	35.61%	N/A	N/A
1	73.42%	84.73%	0.35%
1.5	80.26%	N/A	N/A
2	85.04%	86.53%	2.97%
2.5	87.94%	N/A	N/A
3	90.19%	86.40%	3.01%
4	92.76%	86.20%	3.25%
5	93.50%	85.17%	4.11%

Table A-4 Experimental data of the antioxidant efficacy of the phenolic extracts from avocado byproducts test (ABTS$\bullet^{+}$ assay).

Time (second)	Inhibition Capacity		
	95% aqueous ethanol		
	Peel extract	Seed extract	Negative Control
0	0.00%	0.00%	0.00%
2	28.96%	20.48%	0.15%
8	43.95%	26.91%	0.29%
14	63.11%	29.45%	0.15%
20	78.67%	34.83%	0.00%
26	86.89%	38.57%	0.44%
32	91.79%	41.70%	0.15%
38	94.52%	45.44%	0.15%
44	96.25%	48.13%	1.03%
50	97.41%	50.22%	0.15%
56	97.98%	52.17%	0.29%
62	98.41%	53.96%	0.00%
68	98.56%	55.75%	0.00%
74	98.70%	56.80%	0.15%
80	98.70%	57.85%	0.15%
86	98.85%	58.74%	0.15%
92	99.14%	59.49%	0.15%
98	98.99%	60.09%	0.15%

Table A-4 (cont'd)

104	98.41%	60.54%	0.15%
110	98.27%	60.99%	0.44%
116	98.41%	61.29%	0.15%
122	98.56%	61.58%	0.29%
128	98.70%	61.88%	0.00%
134	98.70%	62.33%	0.00%
140	98.85%	62.48%	0.29%
146	98.99%	62.78%	0.29%
152	99.14%	62.93%	0.29%
158	99.28%	63.08%	0.29%
164	99.57%	63.08%	0.29%
170	99.71%	63.08%	0.29%
176	99.86%	62.93%	0.15%
182	100.00%	63.08%	0.00%

Table A-5 Experimental data of the coating solutions vs. antioxidant efficacy test (ABTS$\bullet^{+}$ assay).

Time (minute)	Inhibition Capacity		
	50% aqueous ethanol		
	PP (laccase)	PP (alkaline)	Negative Control
0	0.00%	0.00%	0.00%
6	15.39%	63.51%	0.58%
12	22.03%	95.81%	1.75%
18	30.13%	100.00%	2.66%
30	37.94%	N/A	3.41%
42	44.84%	N/A	4.29%
54	51.39%	N/A	5.99%
66	63.83%	N/A	7.06%
78	79.38%	N/A	7.06%
90	83.68%	N/A	7.00%

A. Antioxidant efficacy of PP films coated with ethanolic seed extract

Table A-5 (cont'd)

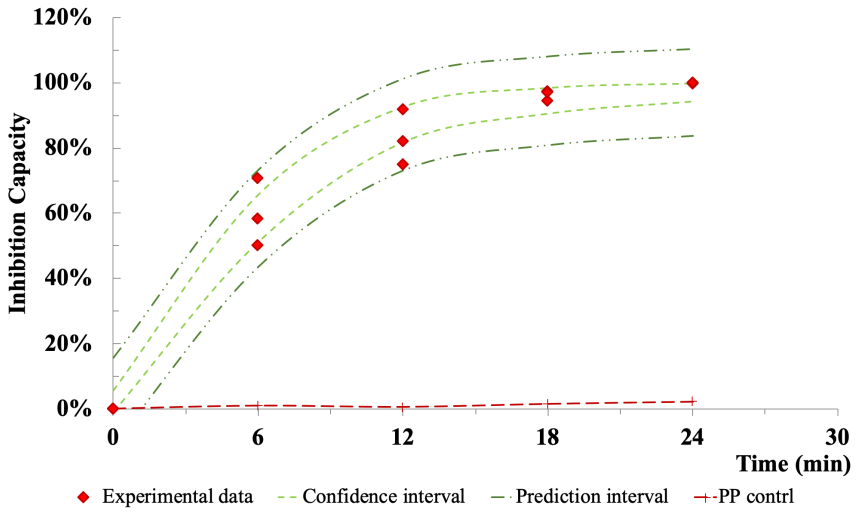
Time (hour)	Inhibition Capacity									
	50% aqueous ethanol									
	DPPH• assay						ABTS•$^{+}$ assay			
	PP	Cntrl	PET	Cntrl	LDPE	Cntrl	PP	Cntrl	PET	Cntrl
0	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
0.1	N/A	N/A	N/A	N/A	N/A	N/A	63.51%	0.58%	82.25%	1.49%
0.2	N/A	N/A	N/A	N/A	N/A	N/A	95.81%	1.75%	99.43%	2.87%
0.3	N/A	N/A	N/A	N/A	N/A	N/A	100.00%	2.66%	100.00%	2.96%
1	84.73%	0.35%	82.50%	1.14%	85.10%	0.23%	N/A	N/A	N/A	N/A
2	86.53%	2.97%	80.52%	2.01%	84.62%	0.69%	N/A	N/A	N/A	N/A
3	86.40%	3.01%	81.48%	3.53%	85.96%	1.87%	N/A	N/A	N/A	N/A
4	86.20%	3.25%	82.02%	3.66%	88.56%	2.94%	N/A	N/A	N/A	N/A
5	85.17%	4.11%	82.66%	4.41%	89.98%	3.61%	N/A	N/A	N/A	N/A

B. Antioxidant activity of coated polymer films tested by DPPH• and ABTS•$^{+}$ assays

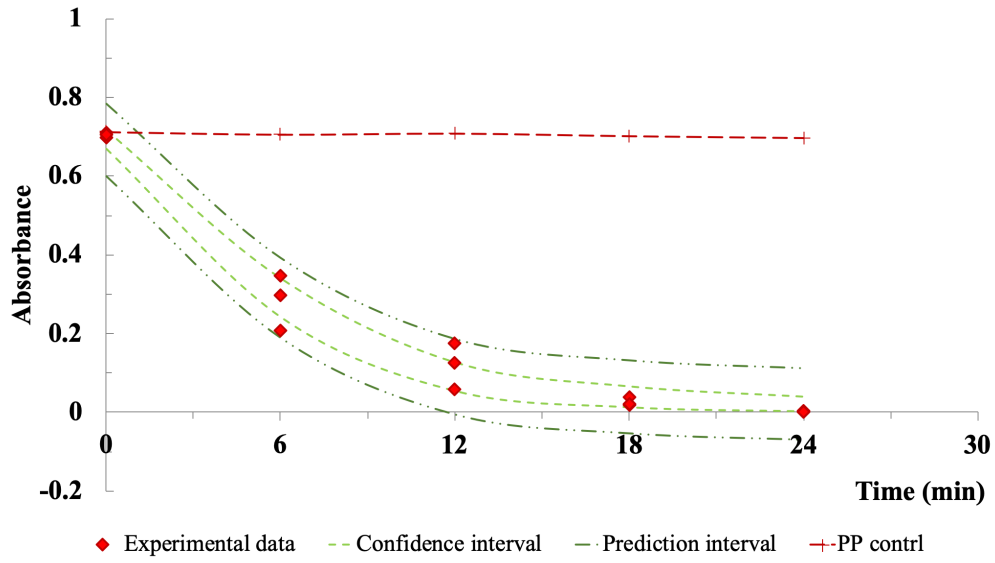
Table A-6 Experimental data of the plastic substrates vs. antioxidant efficacy test (ABTS$\bullet^{+}$ assay).

Time (minute)	Inhibition Capacity			
	10% aqueous ethanol			
	PP	Negative Control	PET	Negative Control
0	0.00%	0.00%	0.00%	0.00%
6	22.19%	0.01%	28.12%	0.12%
12	53.94%	0.40%	56.03%	0.17%
18	74.00%	1.07%	69.89%	0.23%
24	88.64%	1.37%	78.96%	0.30%
30	97.64%	2.06%	88.71%	0.35%
36	99.42%	2.44%	93.50%	0.48%

Appendix B Antioxidant efficiency of coated polymer films (further tests for statistical analysis)



Dose-response model

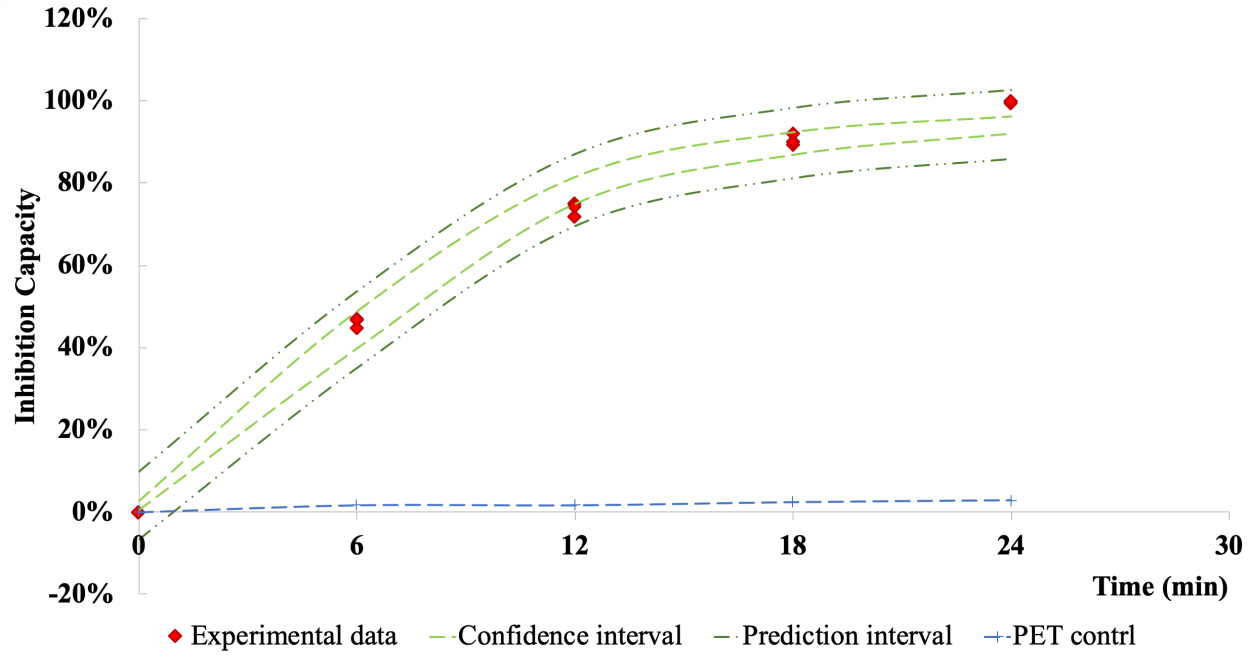


Boltzmann sigmoidal model

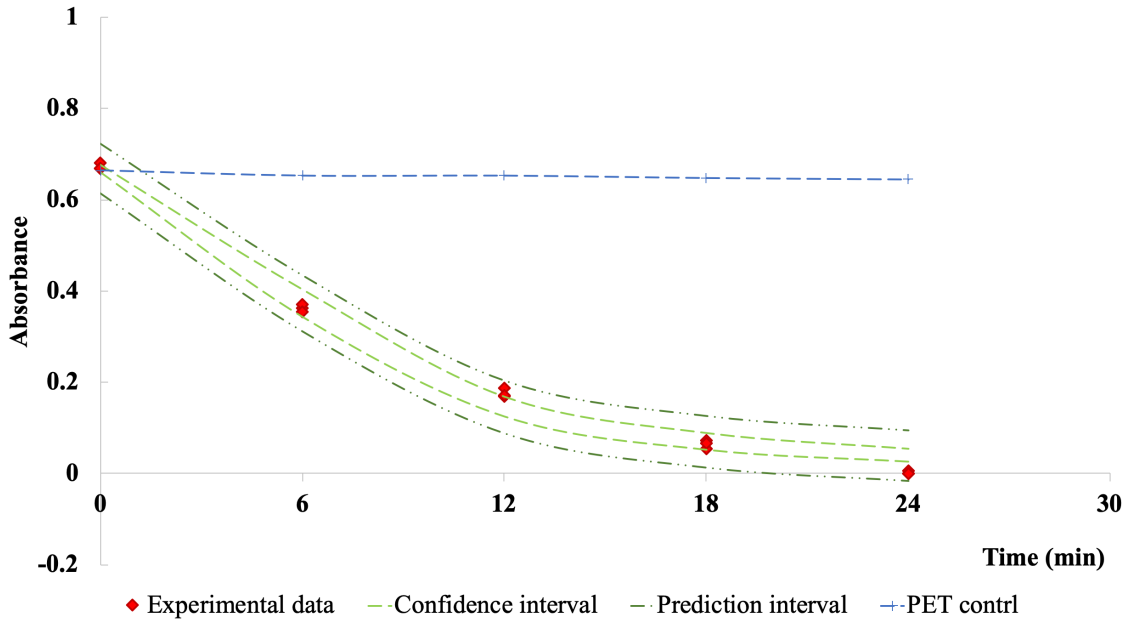
A. PP films coated with the seed extract

Figure B-1 Goodness of fit results estimated by the dose-response and Boltzmann sigmoidal models for the plastic substrates vs. antioxidant efficacy test.

Figure B-1 (cont'd)



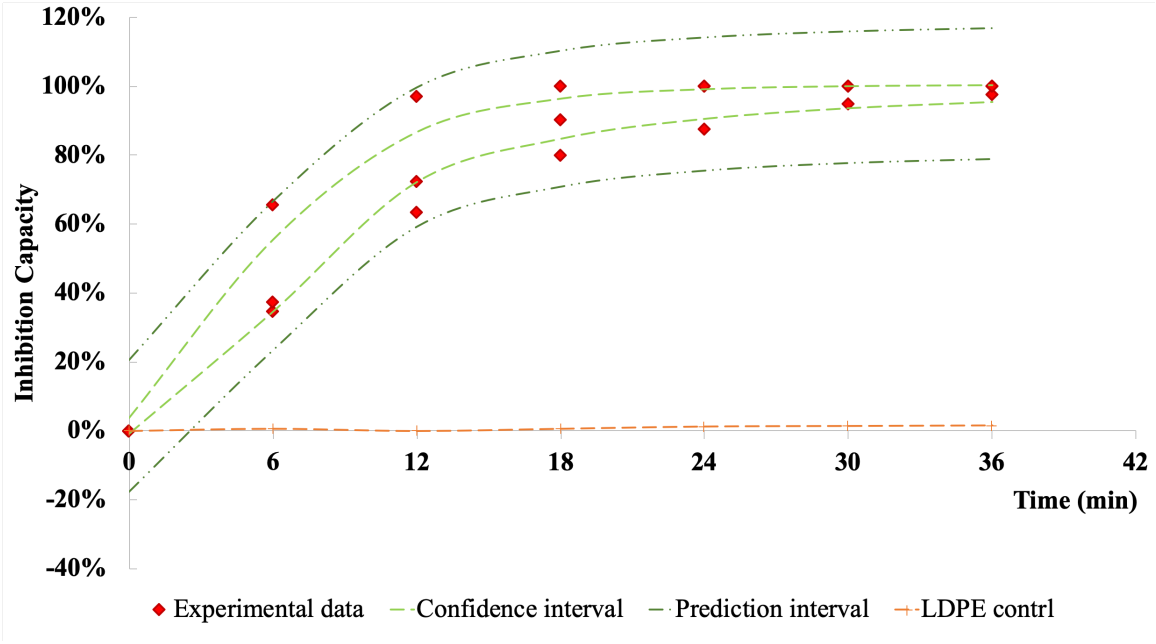
Dose-response model



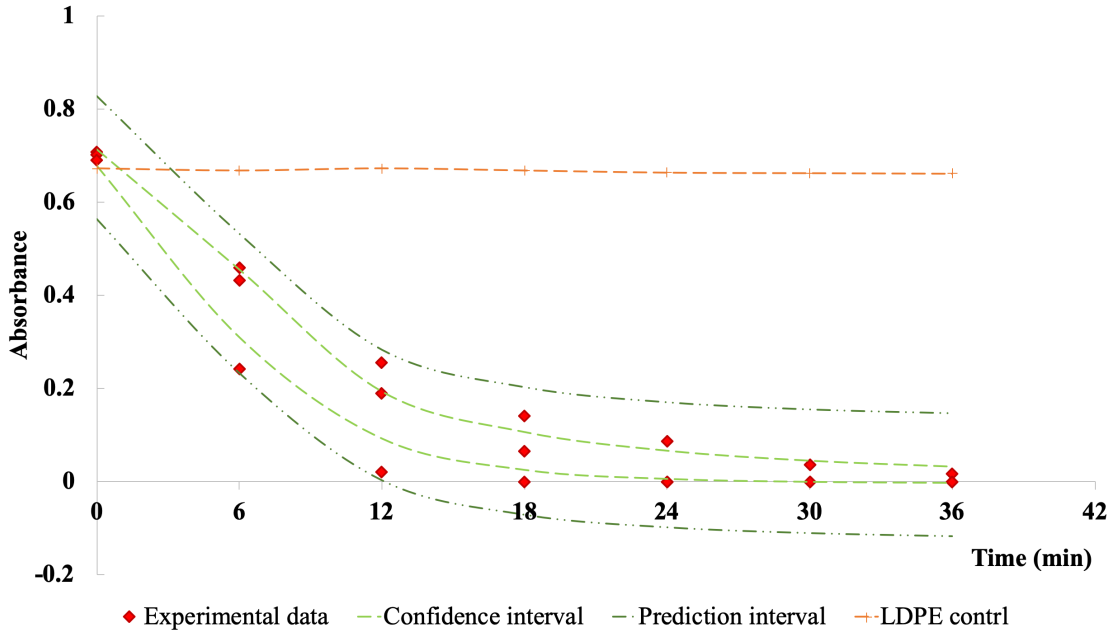
Boltzmann sigmoidal model

B. PET films coated with the seed extract

Figure B-1 (cont'd)



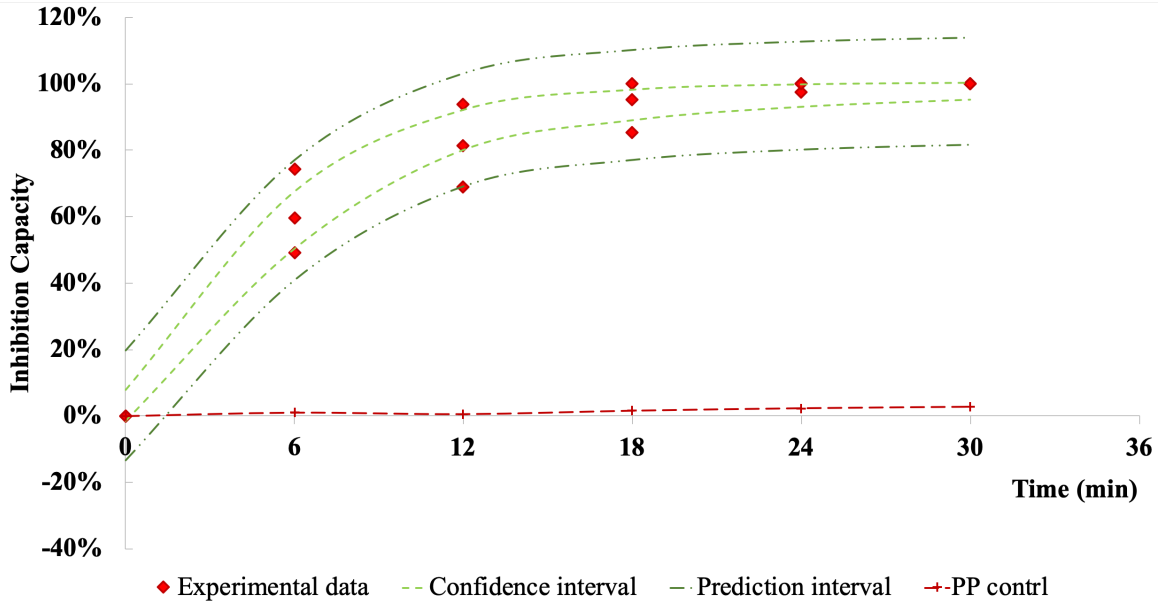
Dose-response model



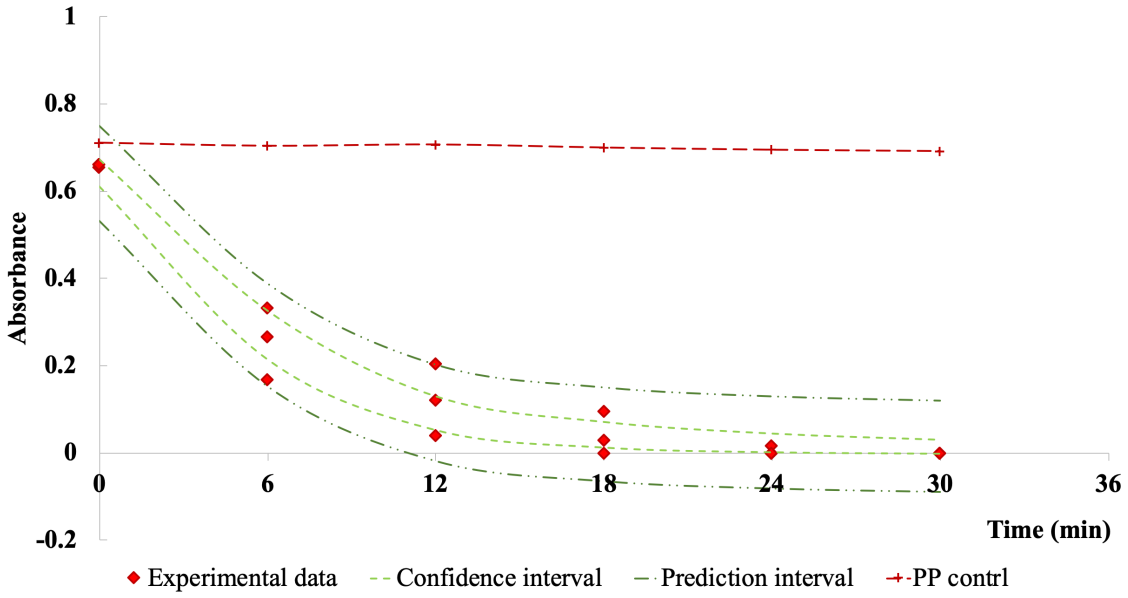
Boltzmann sigmoidal model

C. LDPE films coated with the seed extract

Figure B-1 (cont'd)



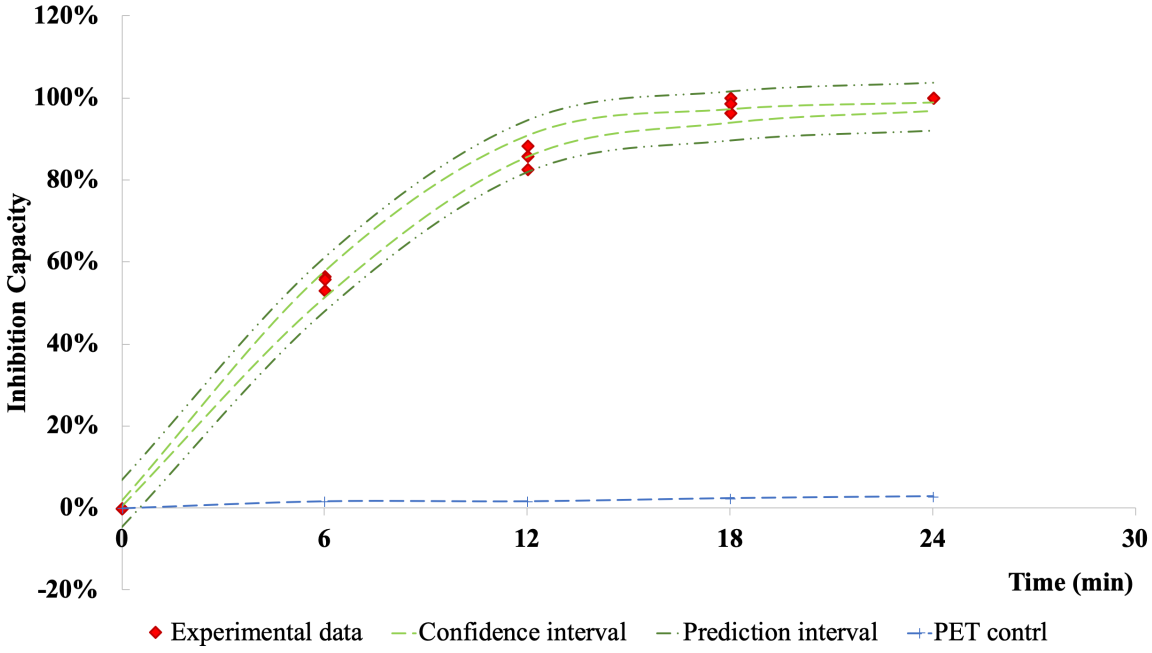
Dose-response model



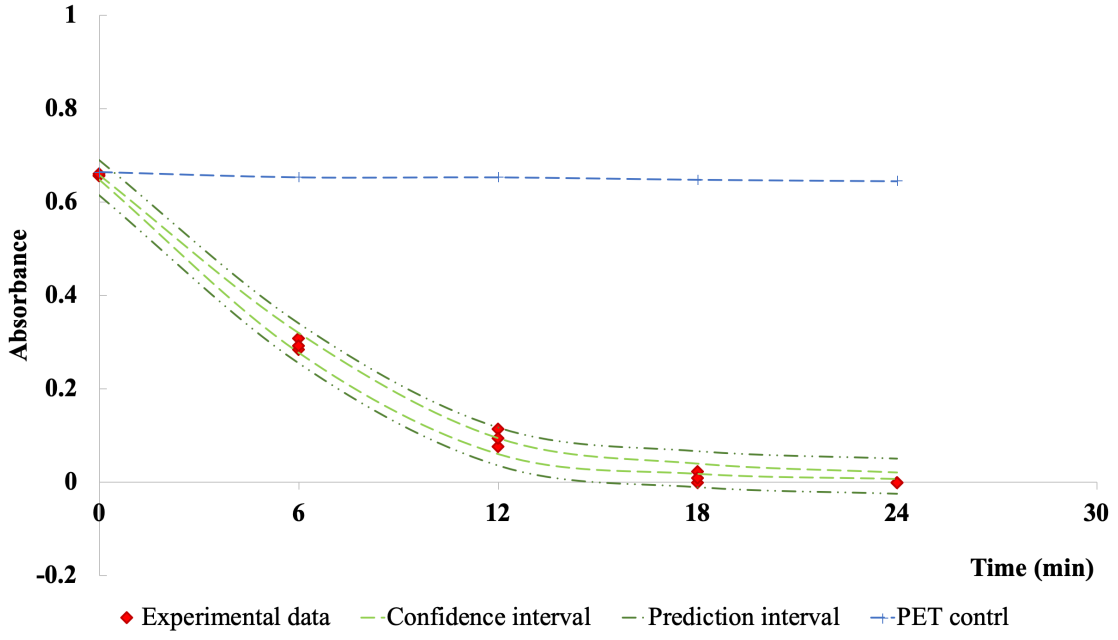
Boltzmann sigmoidal model

D. PP films coated with the peel extract

Figure B-1 (cont'd)



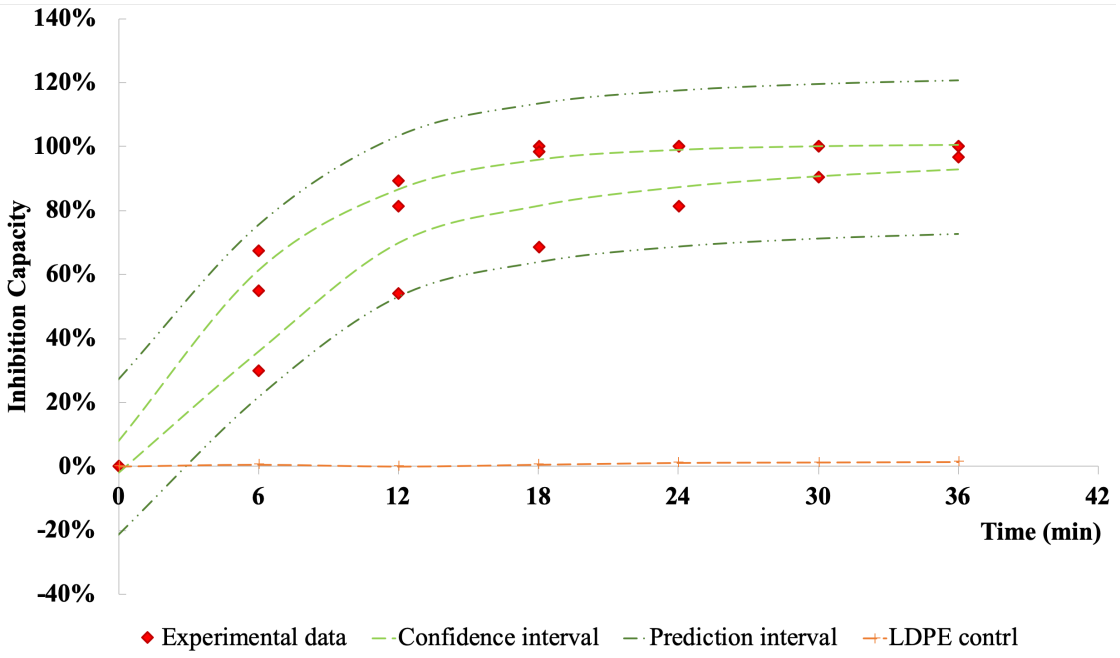
Dose-response model



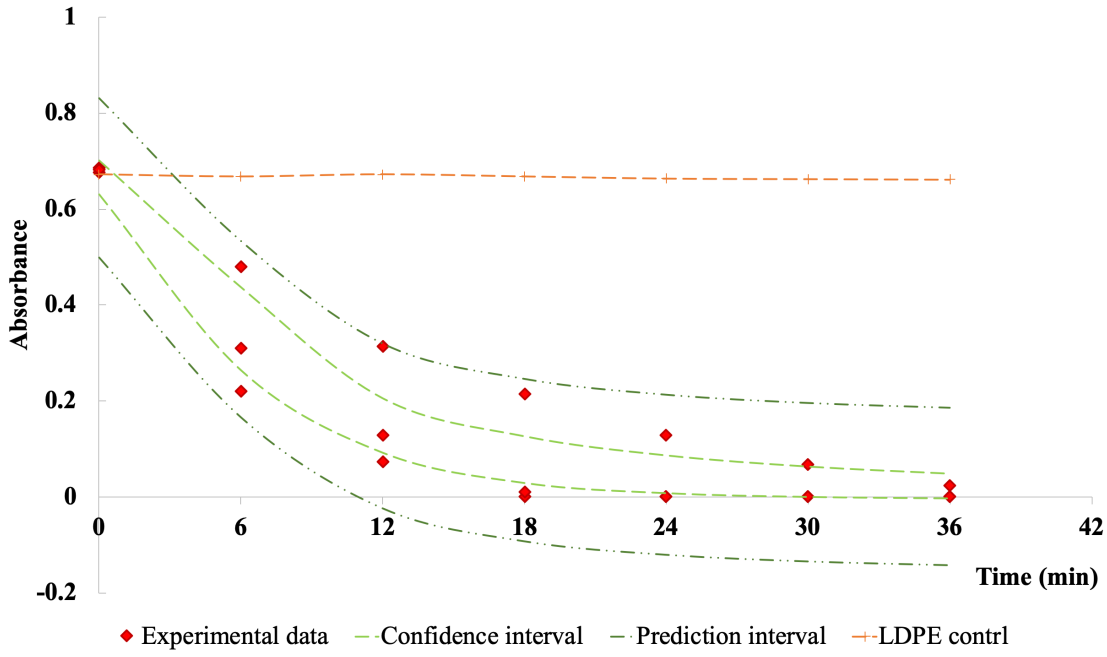
Boltzmann sigmoidal model

E. PET films coated with the peel extract

Figure B-1 (cont'd)



Dose-response model



Boltzmann sigmoidal model

F. LDPE films coated with the peel extract

Table B-1 Fitted regression equations estimated by the dose-response and Boltzmann sigmoidal models for the plastic substrates vs. antioxidant efficacy test.

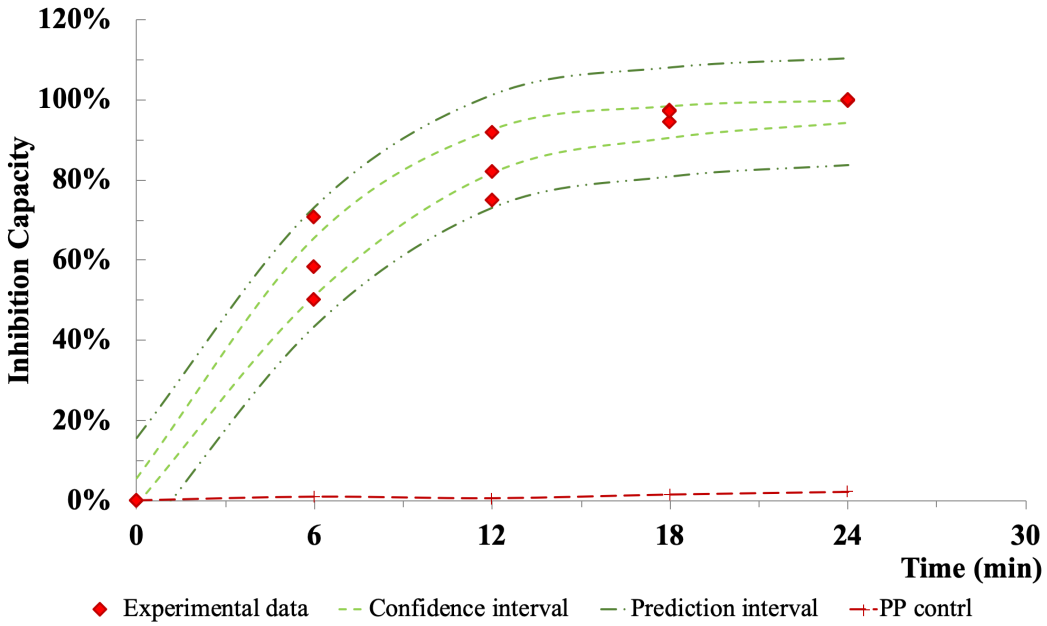
	Dose-response Model $\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(t_0 - t) \times \mathrm{Slope}]}}$	**Boltzmann Sigmoidal Model** $y = \frac{-A'_2}{1 + exp^{(\frac{t - t_0}{\mathrm{Slope}})}} + A'_2$
PP (seed)	$\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(0.713 - t) \times 2.278]}}$	$y = \frac{-0.710}{1 + exp^{(\frac{t - 0.708}{-0.193})}} + 0.710$
PET (seed)	$\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(0.824 - t) \times 2.179]}}$	$y = \frac{-0.680}{1 + exp^{(\frac{t - 0.818}{-0.202})}} + 0.680$
LDPE (seed)	$\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(0.817 - t) \times 2.239]}}$	$y = \frac{-0.707}{1 + exp^{(\frac{t - 0.811}{-0.196})}} + 0.707$
PP (peel)	$\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(0.704 - t) \times 2.107]}}$	$y = \frac{-0.663}{1 + exp^{(\frac{t - 0.701}{-0.207})}} + 0.663$
PET (peel)	$\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(0.748 - t) \times 2.652]}}$	$y = \frac{-0.660}{1 + exp^{(\frac{t - 0.747}{-0.164})}} + 0.660$
LDPE (peel)	$\mathrm{IC}\ (\%) = \frac{100}{1 + 10^{[(0.791 - t) \times 1.930]}}$	$y = \frac{-0.686}{1 + exp^{(\frac{t - 0.788}{-0.227})}} + 0.686$

Table B-2 Experimental data of the plastic substrates vs. antioxidant efficacy test.

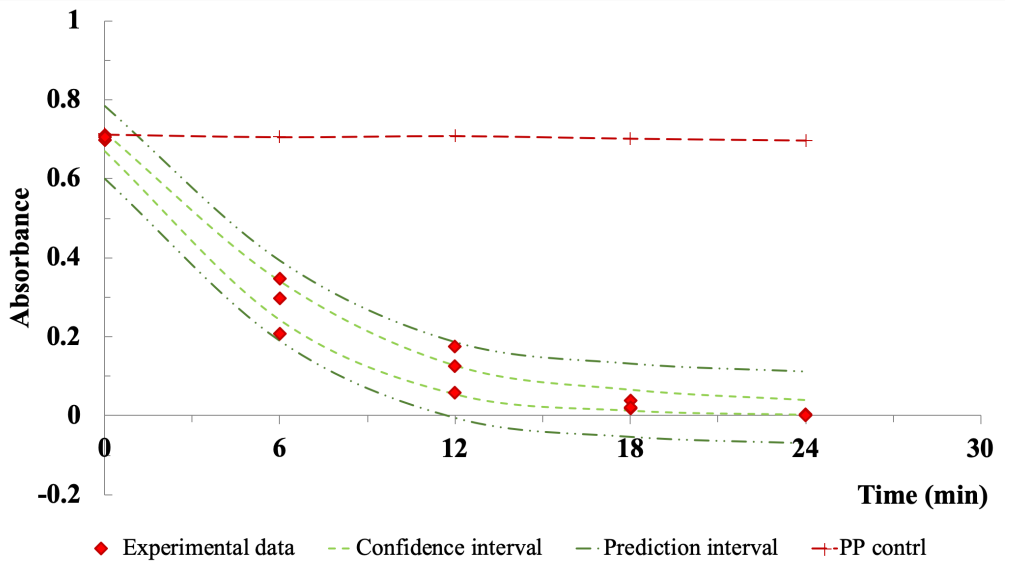
Trial	Sample	0 min	6 min	12 min	18 min	24 min	30 min	36 min
PP (seed)	1	0.696	0.346	0.174	0.037	0.002	N/A	N/A
	2	0.710	0.296	0.057	0.018	0.000	N/A	N/A
	3	0.703	0.206	0.125	0.019	0.000	N/A	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	N/A	N/A
PET (seed)	1	0.680	0.363	0.169	0.054	0.000	N/A	N/A
	2	0.669	0.370	0.188	0.072	0.005	N/A	N/A
	3	0.668	0.355	0.172	0.067	0.000	N/A	N/A
Negative Control		0.664	0.653	0.653	0.648	0.645	N/A	N/A
LDPE (seed)	1	0.707	0.243	0.021	0.000	0.000	0.000	0.000
	2	0.702	0.459	0.256	0.141	0.087	0.036	0.016
	3	0.690	0.432	0.190	0.066	0.000	0.000	0.000
Negative Control		0.672	0.668	0.672	0.668	0.664	0.663	0.662
PP (peel)	1	0.663	0.267	0.123	0.031	0.000	0.000	N/A
	2	0.657	0.334	0.205	0.096	0.017	0.000	N/A
	3	0.663	0.170	0.041	0.000	0.000	0.000	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	0.693	N/A

Table B-2 (cont'd)

PET (peel)	**1**	0.656	0.285	0.077	0.000	0.000	N/A	N/A
	2	0.656	0.308	0.114	0.024	0.000	N/A	N/A
	3	0.660	0.292	0.094	0.009	0.000	N/A	N/A
Negative Control		0.664	0.653	0.653	0.648	0.645	N/A	N/A
LDPE (peel)	**1**	0.677	0.221	0.074	0.000	0.000	0.000	0.000
	2	0.686	0.310	0.129	0.011	0.001	0.000	0.000
	3	0.683	0.480	0.314	0.215	0.129	0.067	0.023
Negative Control		0.672	0.668	0.672	0.668	0.664	0.663	0.662



Dose-response model

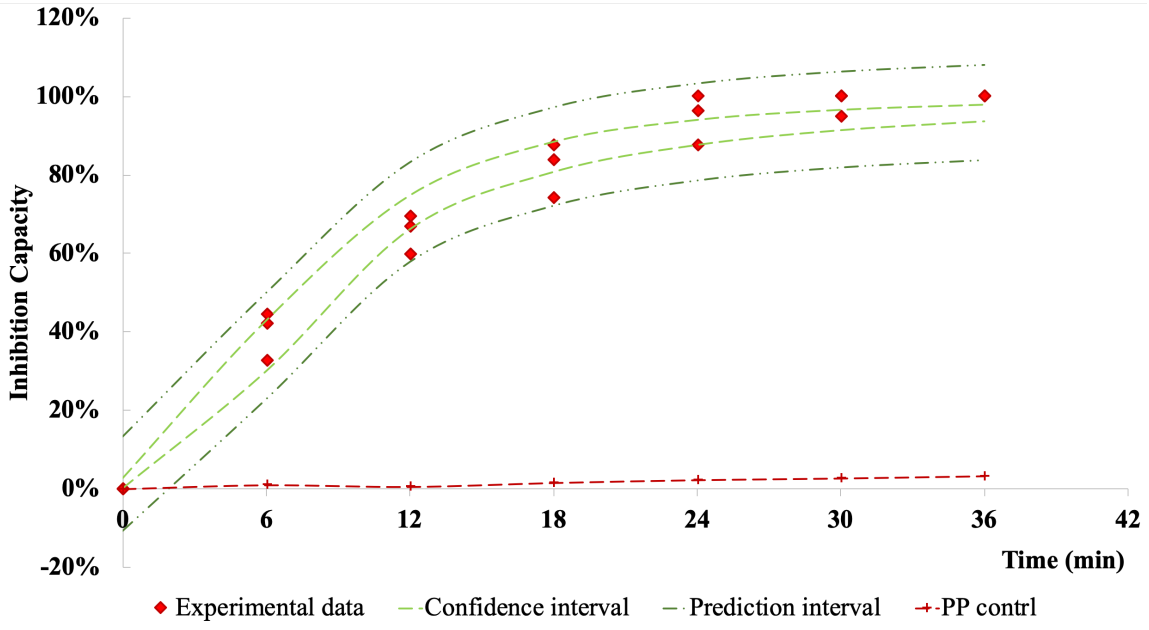


Boltzmann sigmoidal model

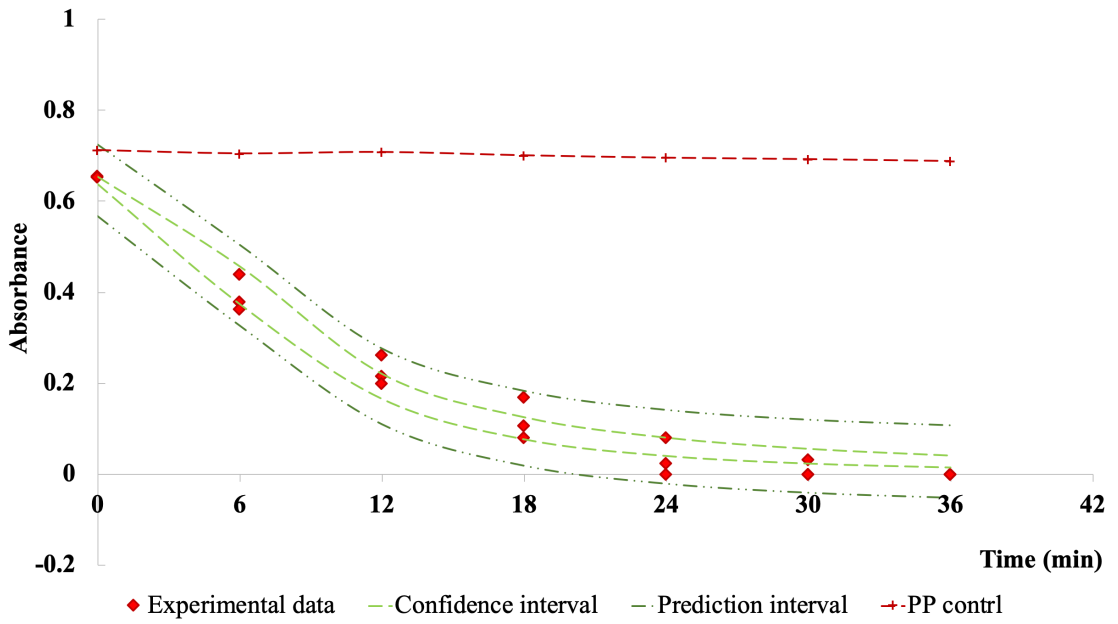
A. PP (<36 hours) films coated with the seed extract

Figure B-2 Goodness of fit results estimated by the dose-response and Boltzmann sigmoidal models for the film storage time vs. antioxidant efficacy test.

Figure B-2 (cont'd)



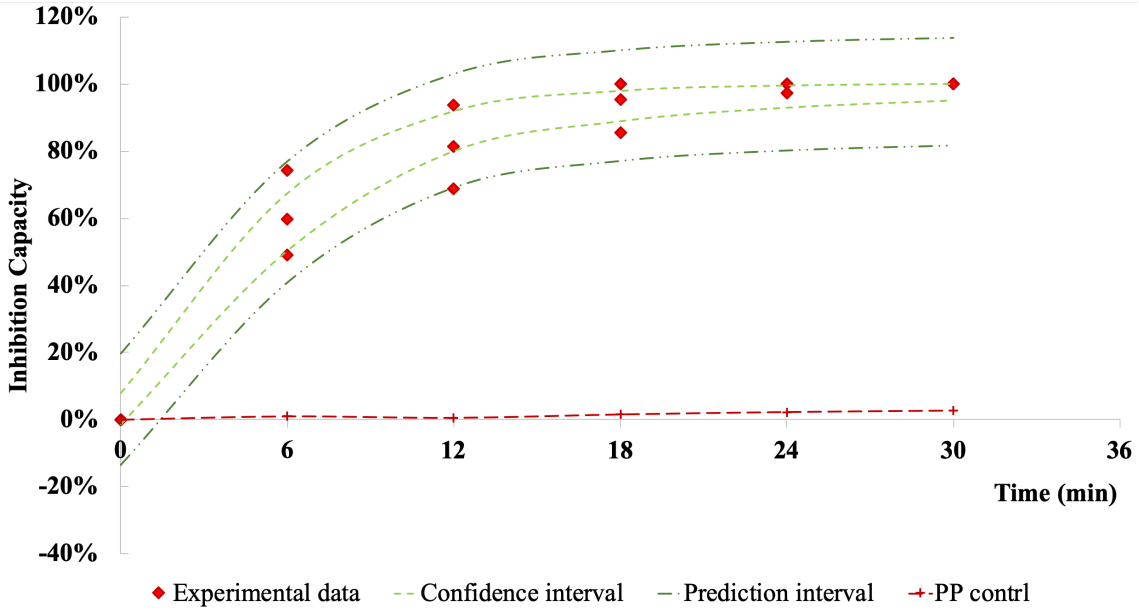
Dose-response model



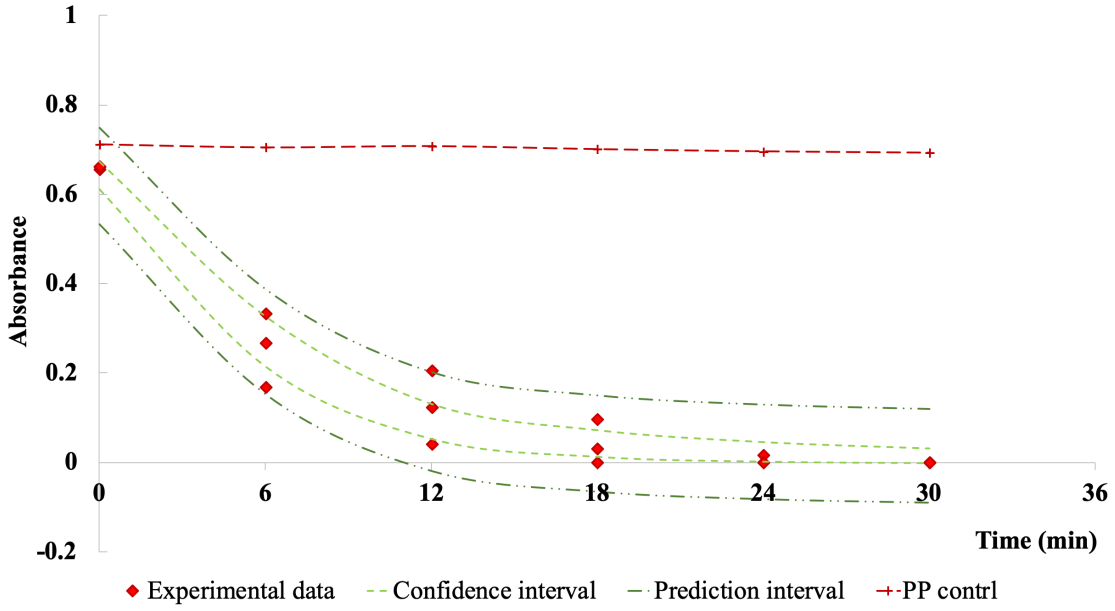
Boltzmann sigmoidal model

B. PP (7 days) films coated with the seed extract

Figure B-2 (cont'd)



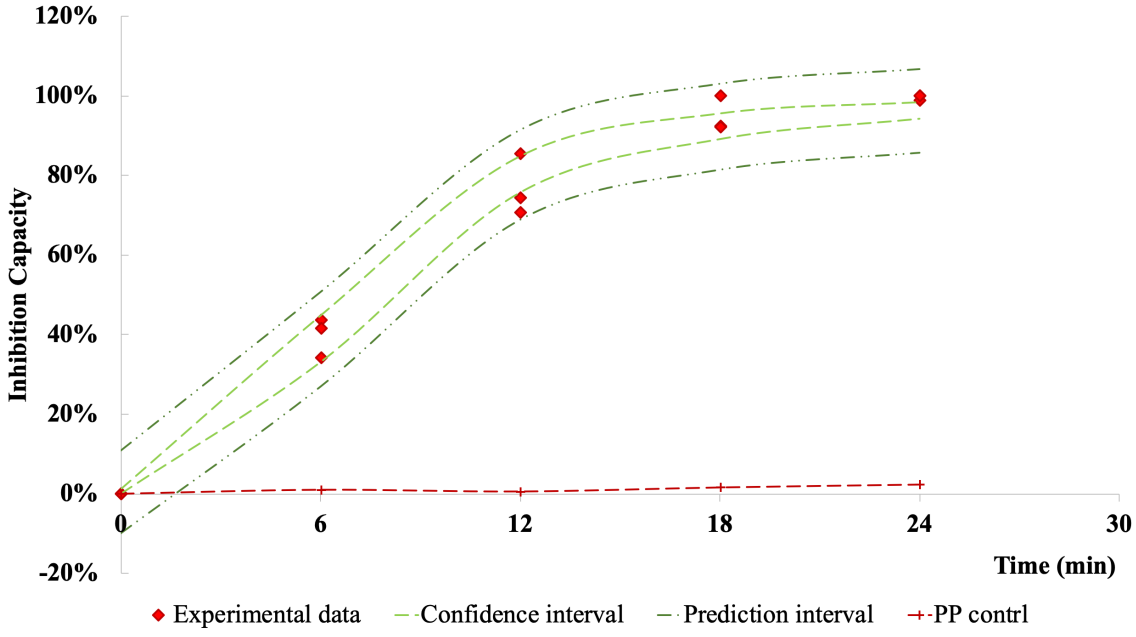
Dose-response model



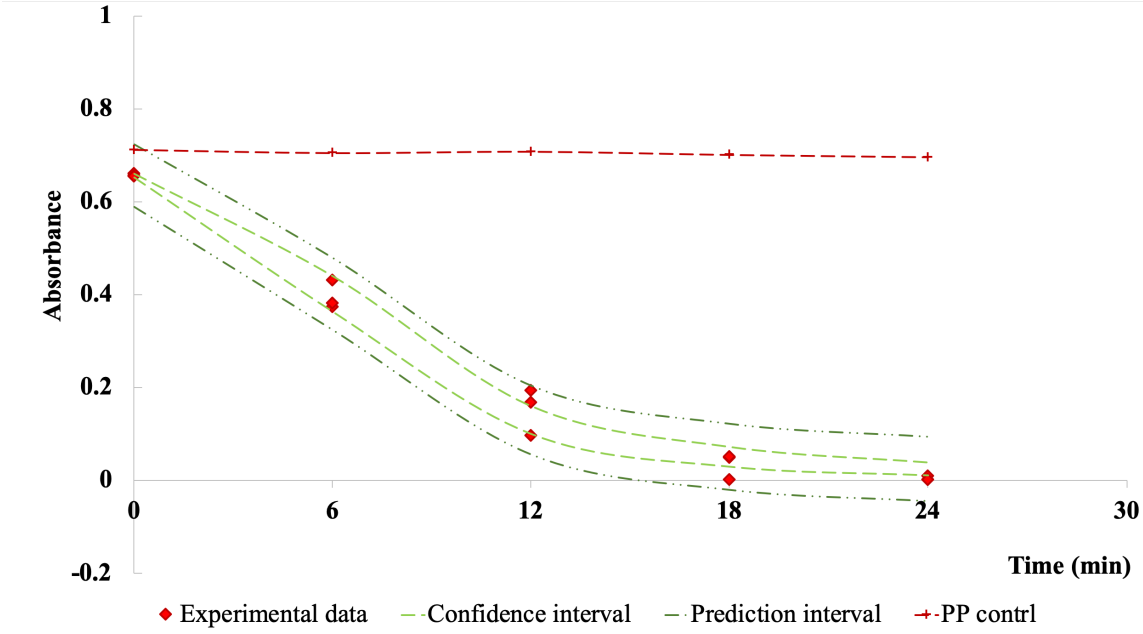
Boltzmann sigmoidal model

C. PP (<36 hours) films coated with the peel extract

Figure B-2 (cont'd)



Dose-response model



Boltzmann sigmoidal model

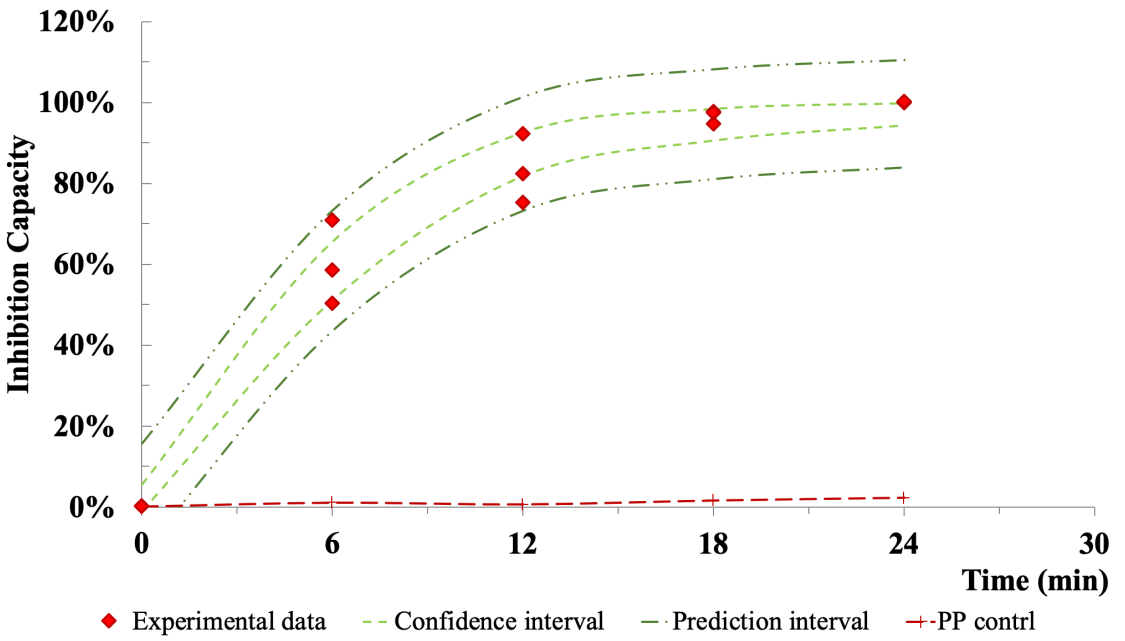
D. PP (7 days) films coated with the peel extract

Table B-3 Fitted regression equations estimated by the dose-response and Boltzmann sigmoidal models for the film storage time vs. antioxidant efficacy test.

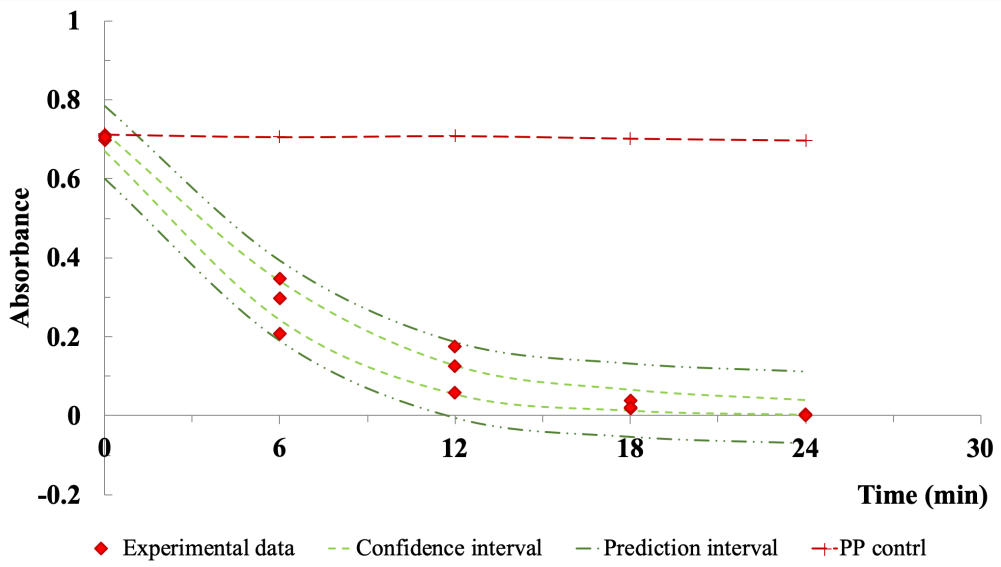
	Dose-response Model $\text{IC}\ (\%) = \frac{100}{1 + 10^{[(t_0 - t) \times \text{Slope}]}}$	**Boltzmann Sigmoidal Model** $y = \frac{-A'_2}{1 + exp^{(\frac{t - t_0}{\text{Slope}})}} + A'_2$
PP (< 36 hours, seed)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.713 - t) \times 2.278]}}$	$y = \frac{-0.710}{1 + exp^{(\frac{t - 0.708}{-0.193})}} + 0.710$
PP (7 days, seed)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.895 - t) \times 2.057]}}$	$y = \frac{-0.656}{1 + exp^{(\frac{t - 0.894}{-0.212})}} + 0.656$
PP (< 36 hours, peel)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.704 - t) \times 2.107]}}$	$y = \frac{-0.663}{1 + exp^{(\frac{t - 0.701}{-0.207})}} + 0.663$
PP (7 days, peel)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.852 - t) \times 2.673]}}$	$y = \frac{-0.659}{1 + exp^{(\frac{t - 0.850}{-0.163})}} + 0.659$

Table B-4 Experimental data of the film storage time vs. antioxidant efficacy test.

Trial	Sample	0 min	6 min	12 min	18 min	24 min	30 min	36 min
PP (< 36 hours, seed)	**1**	0.696	0.346	0.174	0.037	0.002	N/A	N/A
	2	0.710	0.296	0.057	0.018	0.000	N/A	N/A
	3	0.703	0.206	0.125	0.019	0.000	N/A	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	N/A	N/A
PP (7 days, seed)	**1**	0.654	0.379	0.217	0.081	0.000	0.000	0.000
	2	0.656	0.364	0.201	0.107	0.024	0.000	0.000
	3	0.654	0.441	0.263	0.170	0.082	0.033	0.000
Negative Control		0.712	0.705	0.708	0.701	0.696	0.693	0.689
PP (< 36 hours, peel)	**1**	0.663	0.267	0.123	0.031	0.000	0.000	N/A
	2	0.657	0.334	0.205	0.096	0.017	0.000	N/A
	3	0.663	0.170	0.041	0.000	0.000	0.000	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	0.693	N/A
PP (7 days, peel)	**1**	0.656	0.431	0.192	0.049	0.008	N/A	N/A
	2	0.659	0.372	0.096	0.000	0.000	N/A	N/A
	3	0.653	0.381	0.167	0.051	0.000	N/A	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	N/A	N/A



Dose-response model

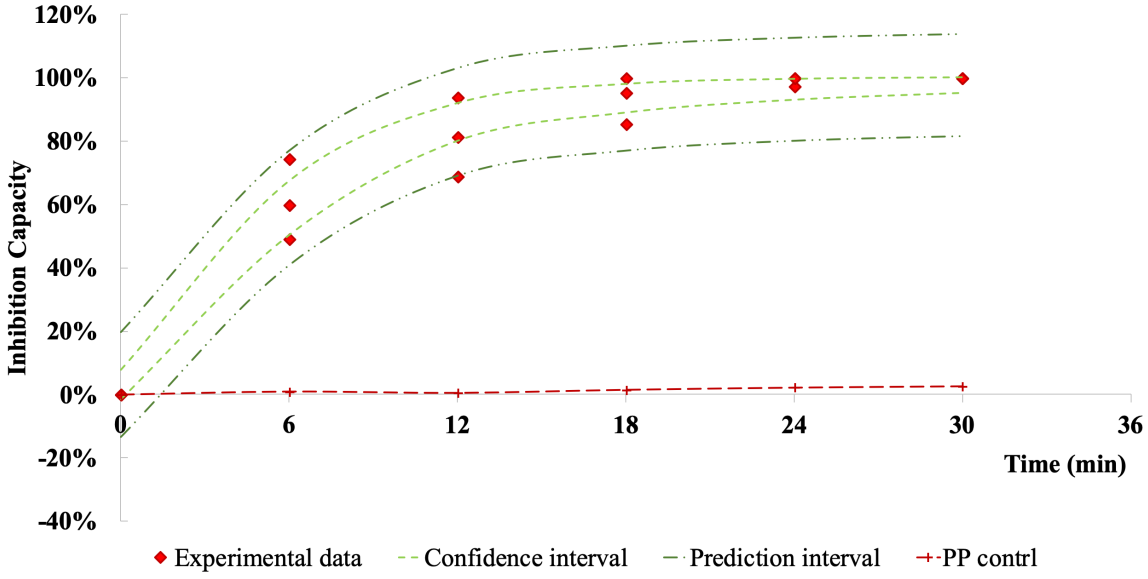


Boltzmann sigmoidal model

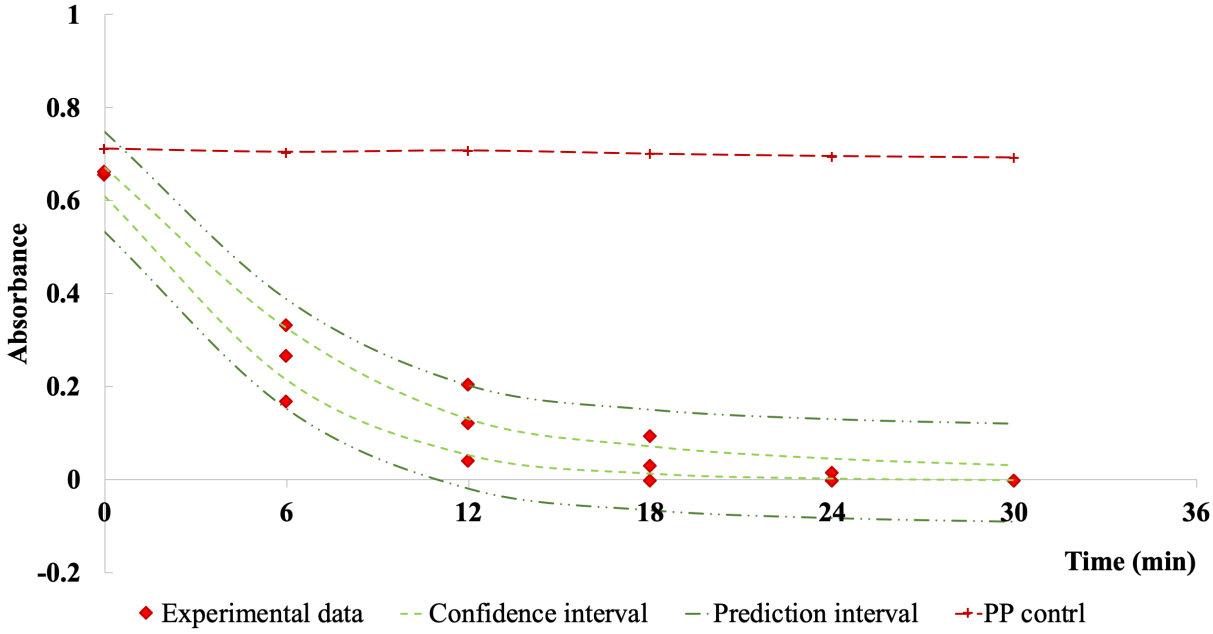
A. PP (100% seed)

Figure B-3 Goodness of fit results estimated by the dose-response and Boltzmann sigmoidal models for the extract ratio vs. antioxidant efficacy test.

Figure B-3 (cont'd)



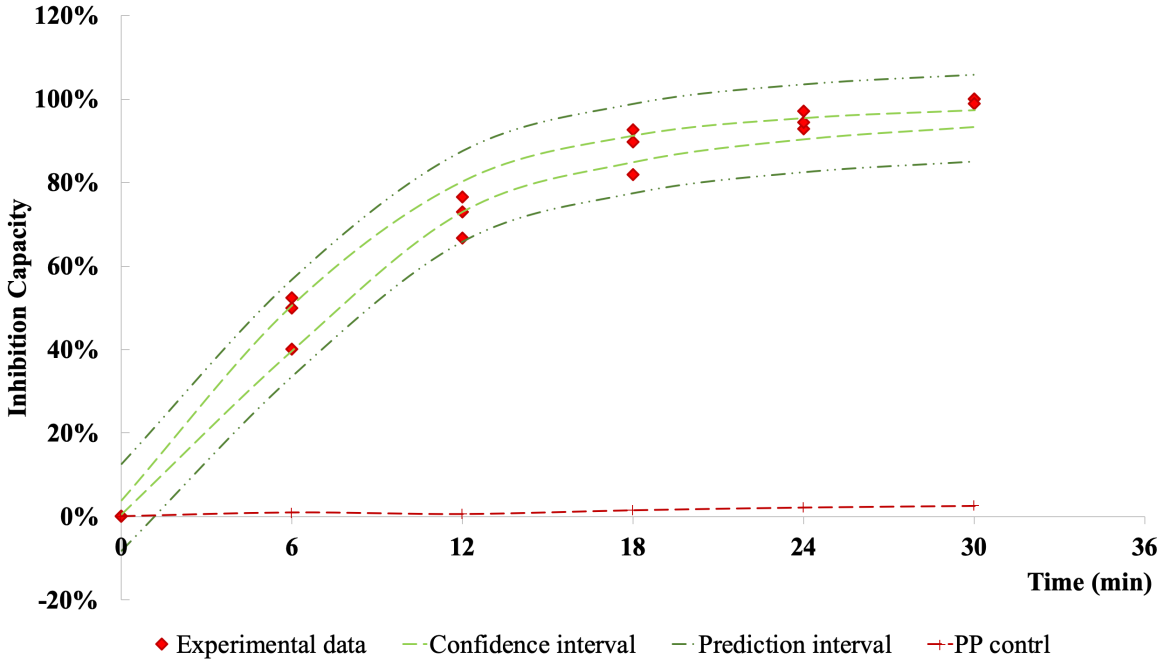
Dose-response model



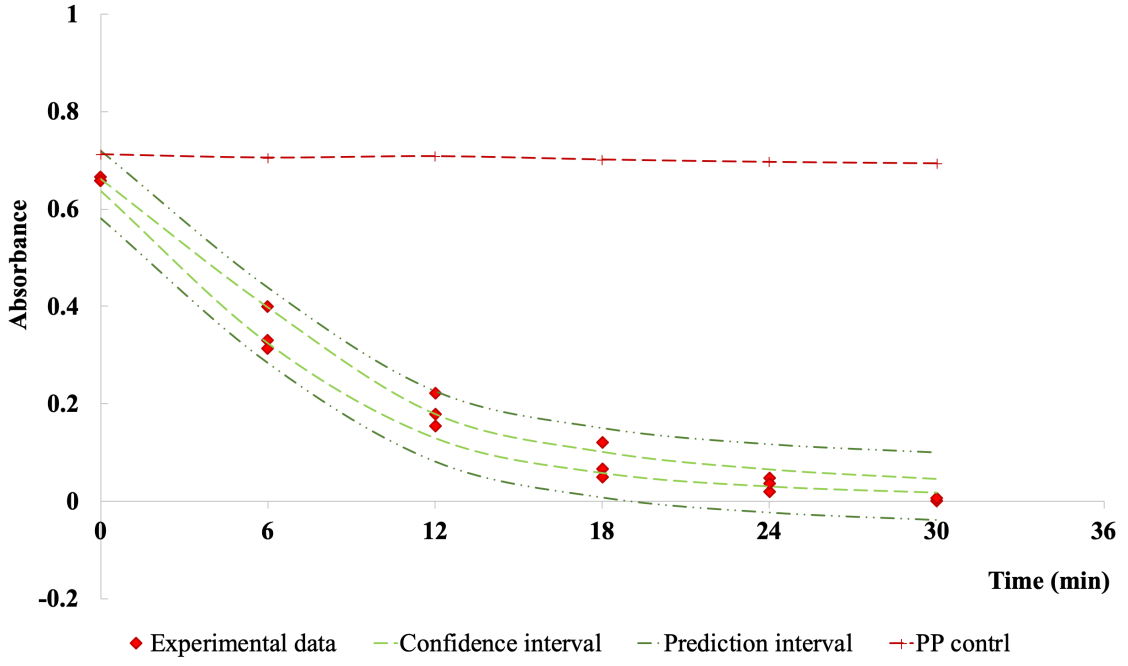
Boltzmann sigmoidal model

B. PP (100% peel)

Figure B-3 (cont'd)



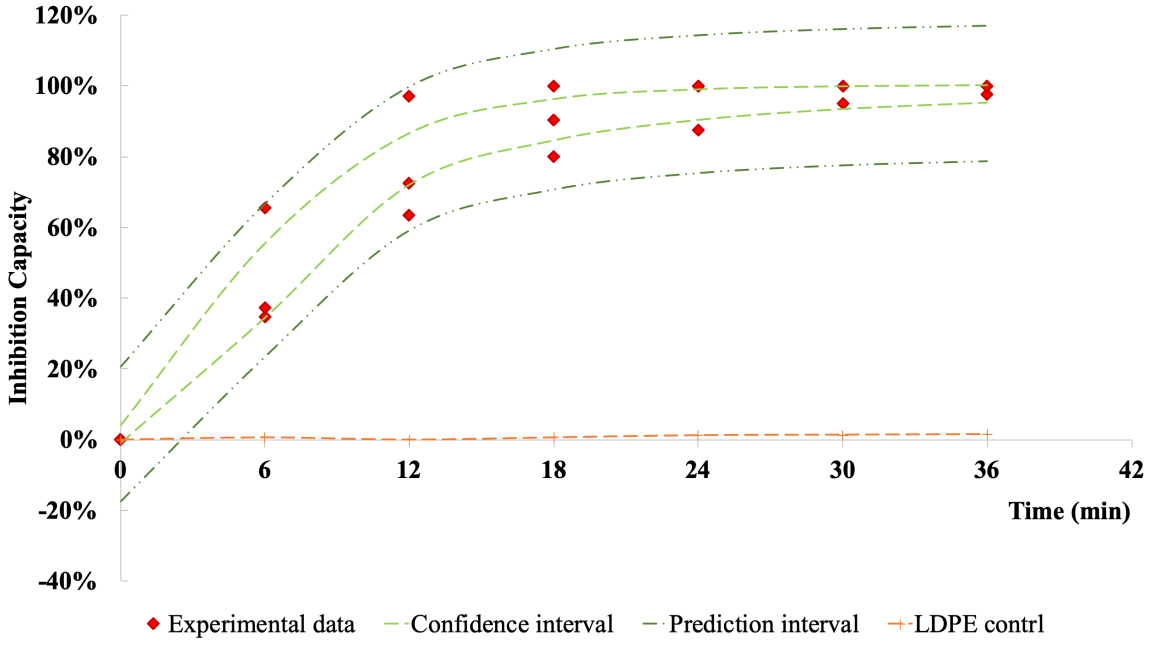
Dose-response model



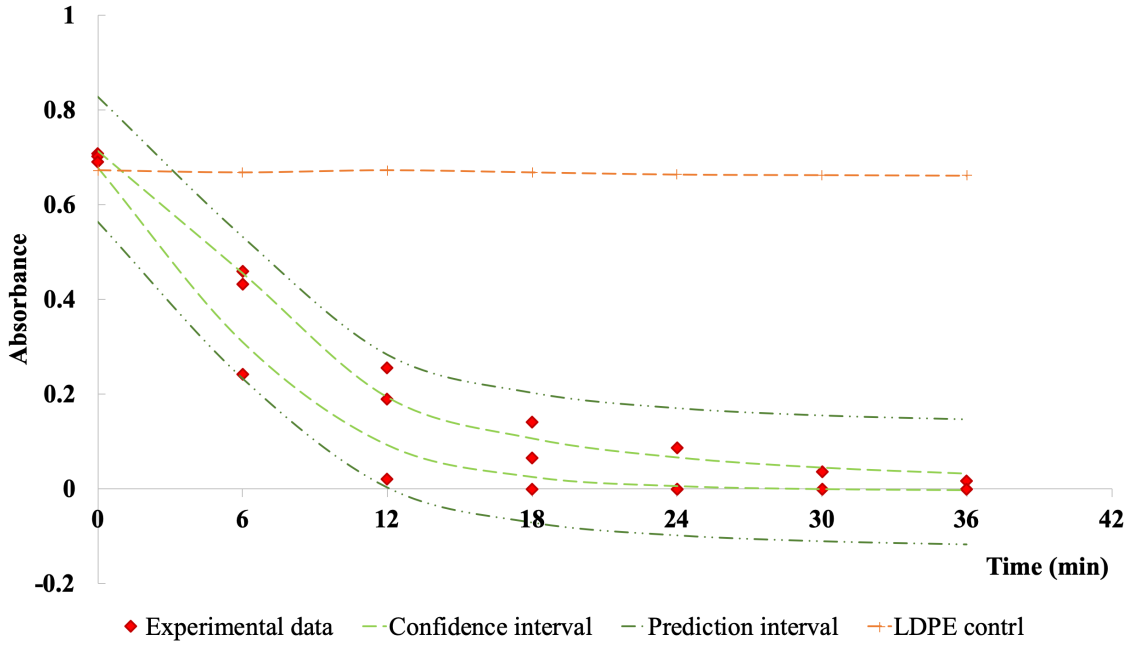
Boltzmann sigmoidal model

C. PP (50% seed + 50% peel)

Figure B-3 (cont'd)



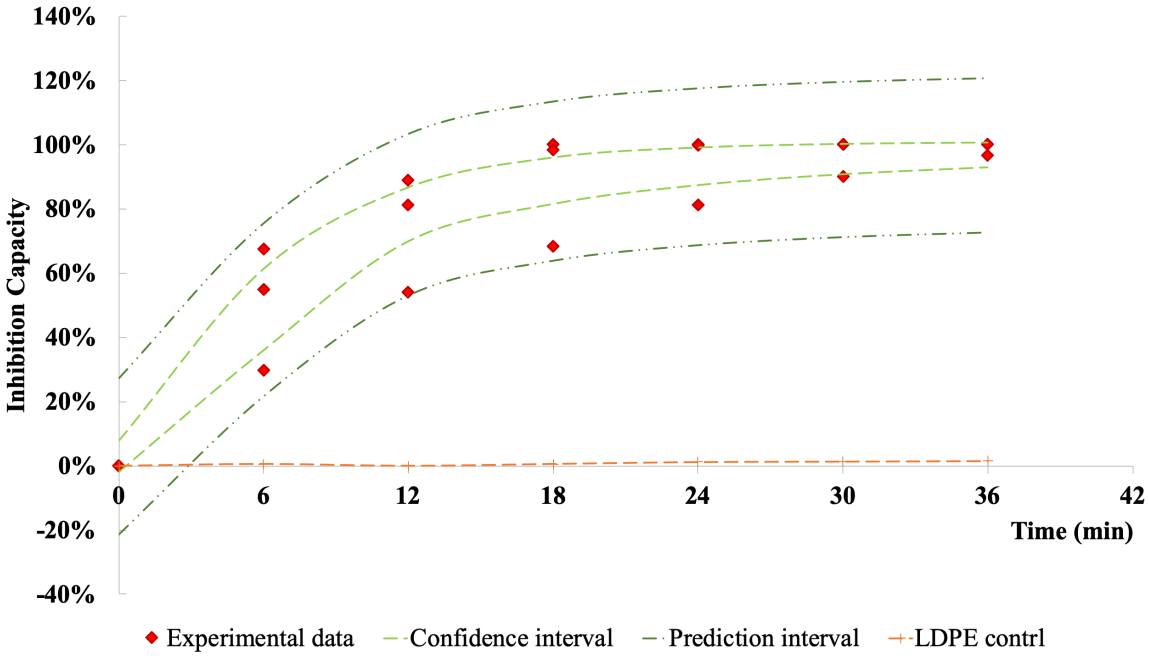
Dose-response model



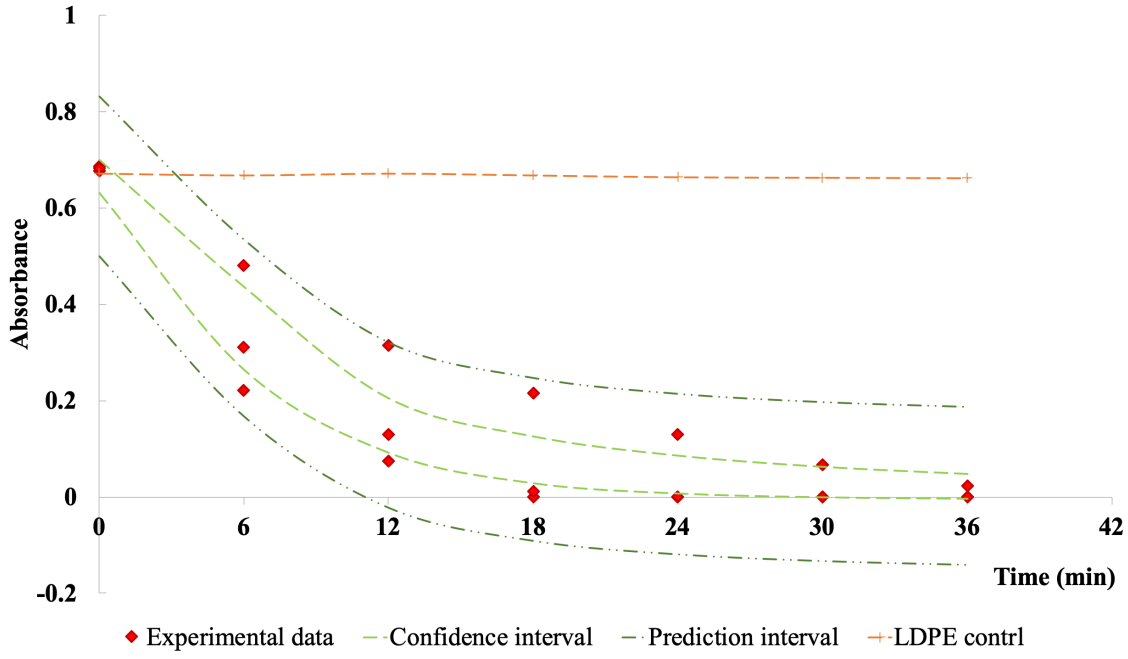
Boltzmann sigmoidal model

D. LDPE (100% seed)

Figure B-3 (cont'd)



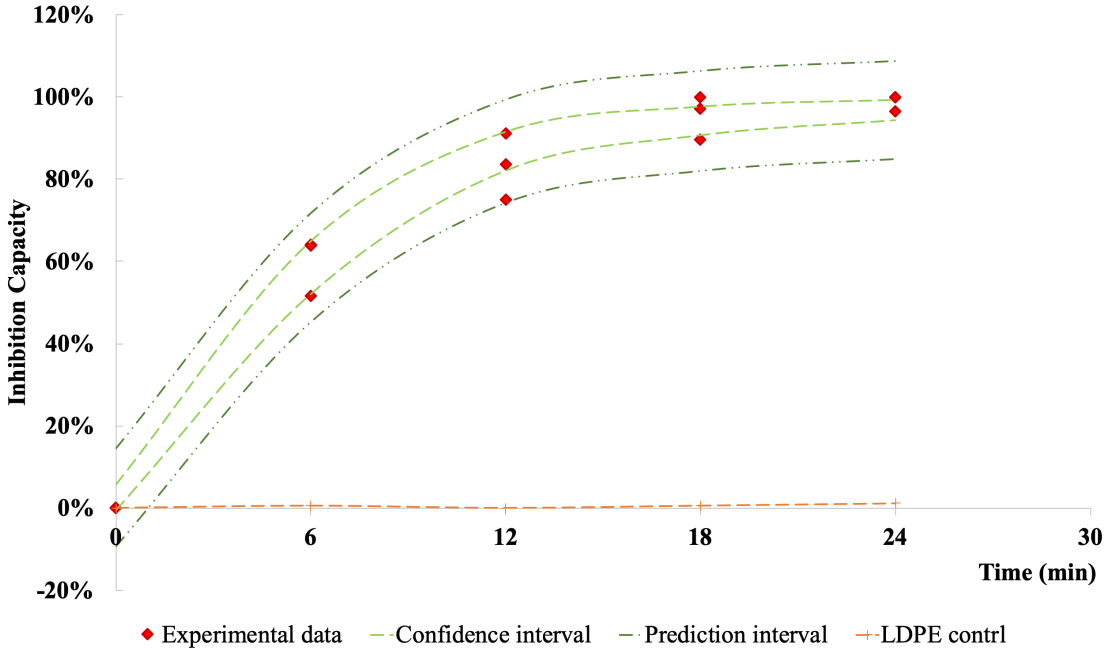
Dose-response model



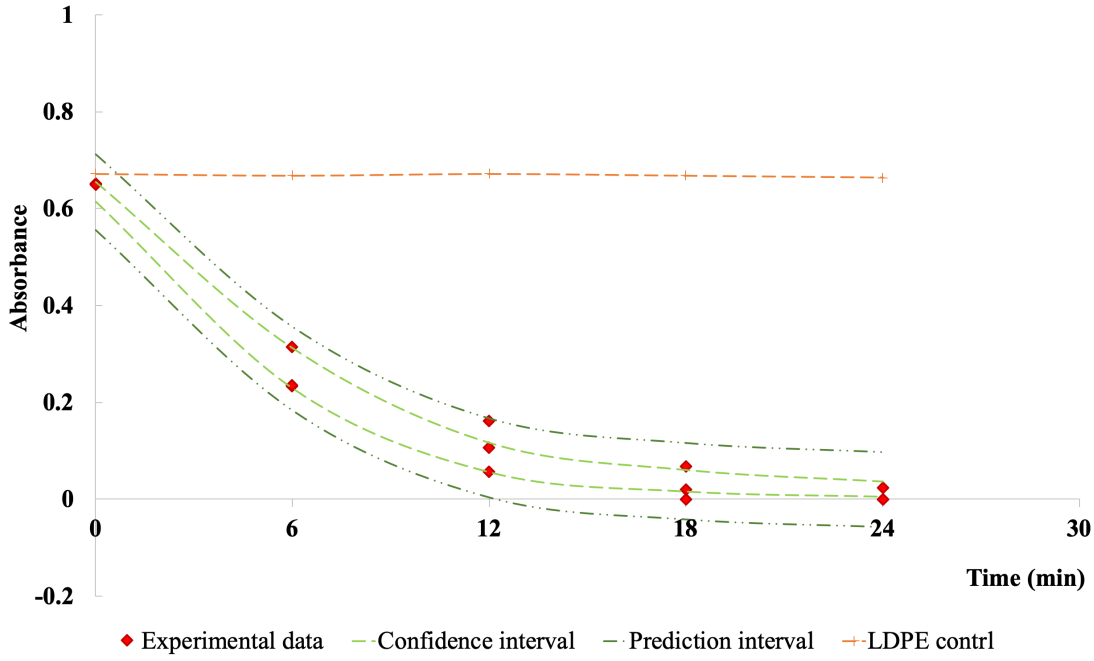
Boltzmann sigmoidal model

E. LDPE (100% peel)

Figure B-3 (cont'd)



Dose-response model



Boltzmann sigmoidal model

F. LDPE (50% seed + 50% peel)

Table B-5 Fitted regression equations estimated by the dose-response and Boltzmann sigmoidal models for the extract ratio vs. antioxidant efficacy test.

	Dose-response Model $\text{IC}\ (\%) = \frac{100}{1 + 10^{[(t_0 - t) \times \text{Slope}]}}$	**Boltzmann Sigmoidal Model** $y = \frac{-A'_2}{1 + exp^{(\frac{t - t_0}{\text{Slope}})}} + A'_2$
PP (100% seed)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.713 - t) \times 2.278]}}$	$y = \frac{-0.710}{1 + exp^{(\frac{t - 0.708}{-0.193})}} + 0.710$
PP (100% peel)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.704 - t) \times 2.107]}}$	$y = \frac{-0.663}{1 + exp^{(\frac{t - 0.701}{-0.207})}} + 0.663$
PP (50% seed + 50% peel)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.820 - t) \times 1.996]}}$	$y = \frac{-0.666}{1 + exp^{(\frac{t - 0.816}{-0.220})}} + 0.666$
LDPE (100% seed)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.817 - t) \times 2.239]}}$	$y = \frac{-0.707}{1 + exp^{(\frac{t - 0.811}{-0.196})}} + 0.707$
LDPE (100% peel)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.791 - t) \times 1.930]}}$	$y = \frac{-0.686}{1 + exp^{(\frac{t - 0.788}{-0.227})}} + 0.686$
LDPE (50% seed + 50% peel)	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.711 - t) \times 2.227]}}$	$y = \frac{-0.651}{1 + exp^{(\frac{t - 0.711}{-0.195})}} + 0.651$

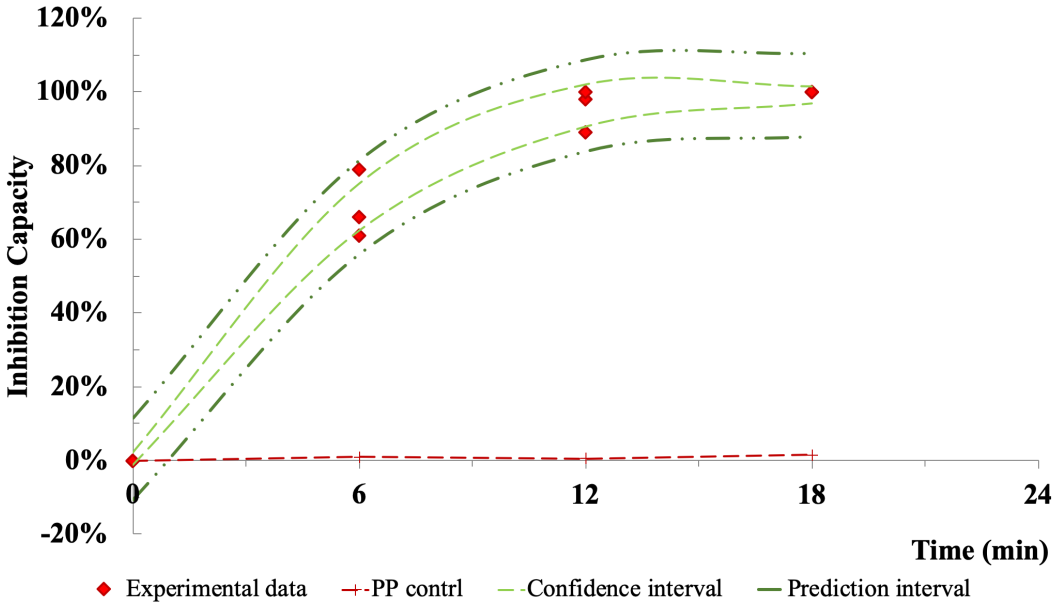
Table B-6 Experimental data of the extract ratio vs. antioxidant efficacy test.

Trial	Sample	0 min	6 min	12 min	18 min	24 min	30 min	36 min
PP (100% seed)	**1**	0.696	0.346	0.174	0.037	0.002	N/A	N/A
	2	0.710	0.296	0.057	0.018	0.000	N/A	N/A
	3	0.703	0.206	0.125	0.019	0.000	N/A	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	N/A	N/A
PP (100% peel)	**1**	0.663	0.267	0.123	0.031	0.000	0.000	N/A
	2	0.657	0.334	0.205	0.096	0.017	0.000	N/A
	3	0.663	0.170	0.041	0.000	0.000	0.000	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	0.693	N/A
PP (50% seed + 50% peel)	**1**	0.658	0.330	0.154	0.067	0.019	0.000	N/A
	2	0.666	0.399	0.222	0.121	0.047	0.000	N/A
	3	0.658	0.313	0.178	0.049	0.036	0.007	N/A
Negative Control		0.712	0.705	0.708	0.701	0.696	0.693	N/A
LDPE (100% seed)	**1**	0.707	0.243	0.021	0.000	0.000	0.000	0.000
	2	0.702	0.459	0.256	0.141	0.087	0.036	0.016
	3	0.690	0.432	0.190	0.066	0.000	0.000	0.000
Negative Control		0.672	0.668	0.672	0.668	0.664	0.663	0.662

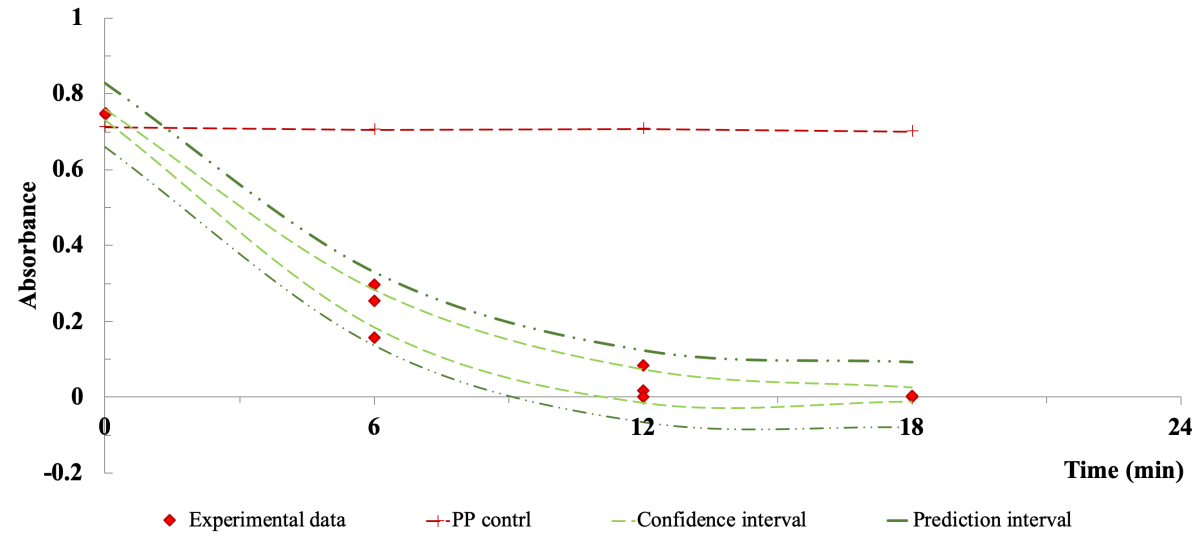
Table B-6 (cont'd)

LDPE (100% peel)	**1**	0.677	0.221	0.074	0.000	0.000	0.000	0.000
	2	0.686	0.310	0.129	0.011	0.001	0.000	0.000
	3	0.683	0.480	0.314	0.215	0.129	0.067	0.023
Negative Control		0.672	0.668	0.672	0.668	0.664	0.663	0.662
LDPE (50% seed + 50% peel)	**1**	0.651	0.315	0.162	0.068	0.023	N/A	N/A
	2	0.651	0.234	0.106	0.019	0.000	N/A	N/A
	3	0.650	0.235	0.057	0.000	0.000	N/A	N/A
Negative Control		0.672	0.668	0.672	0.668	0.664	N/A	N/A

Appendix C Mode of antioxidant activity of coated polymer films



Dose-response model

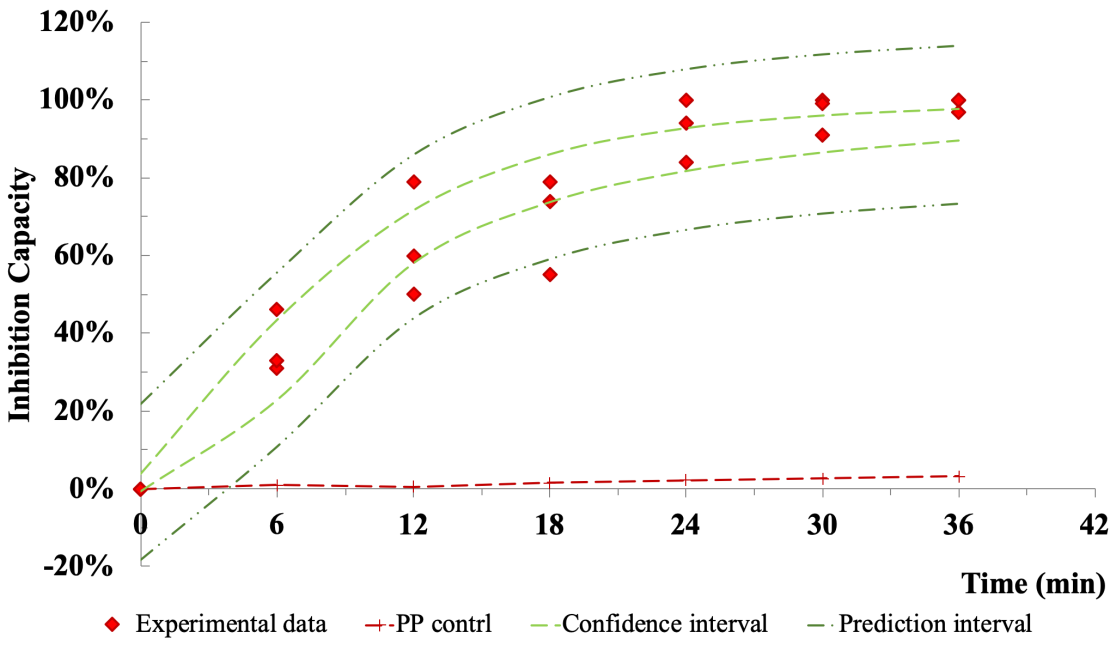


Boltzmann sigmoidal model

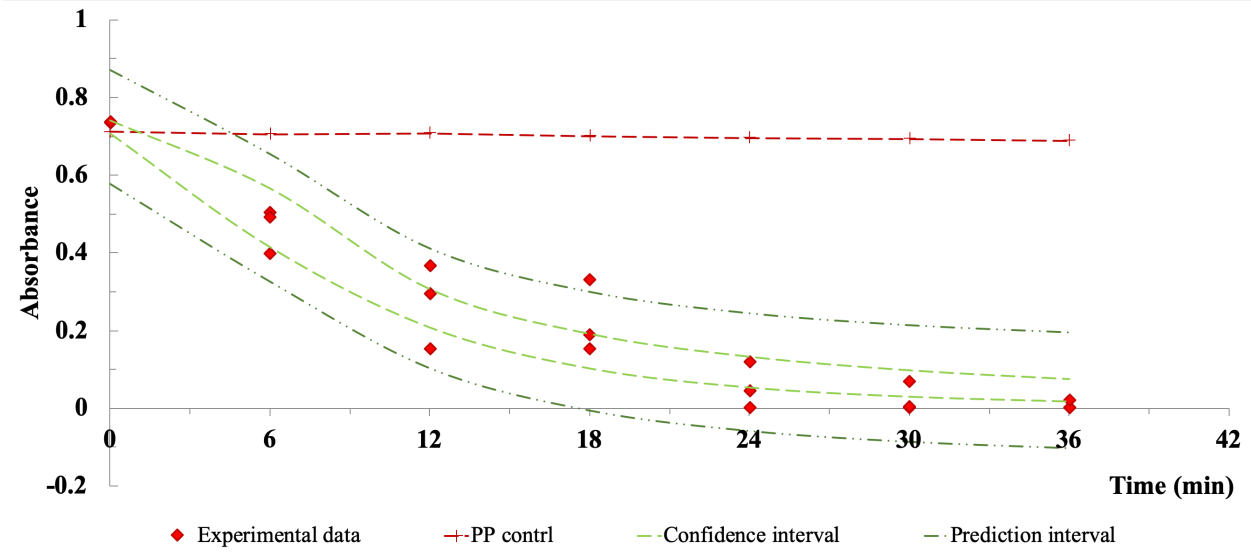
A. Trial 1

Figure C-1 Goodness of fit results estimated by the dose-response and Boltzmann sigmoidal models for the antioxidative recoverability test.

Figure C-1 (cont'd)



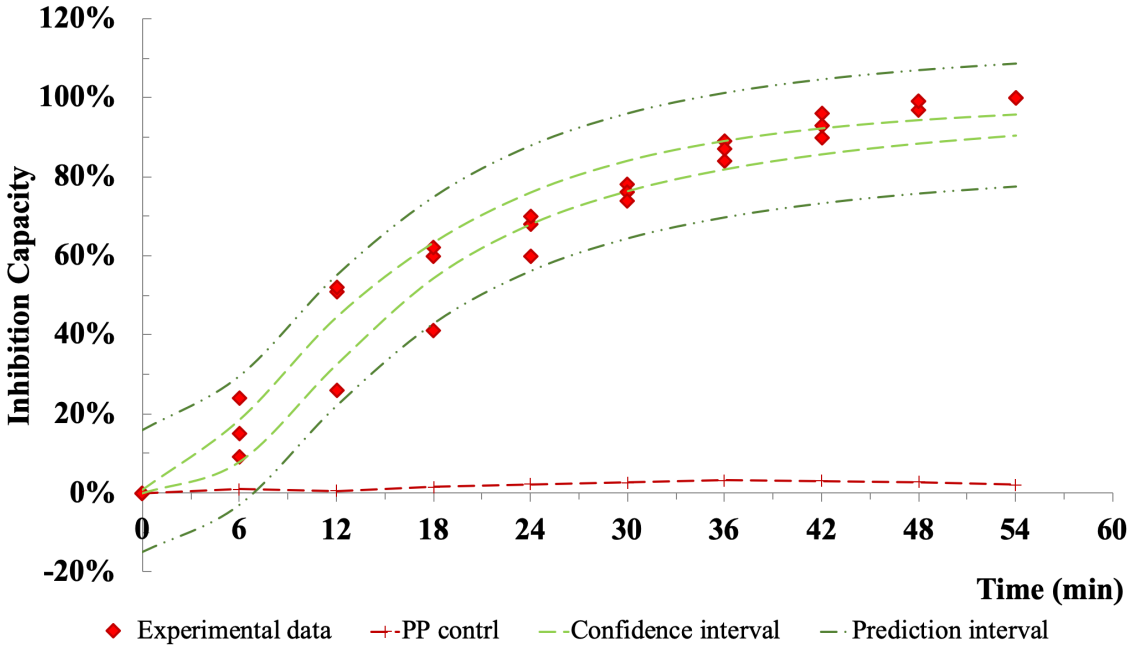
Dose-response model



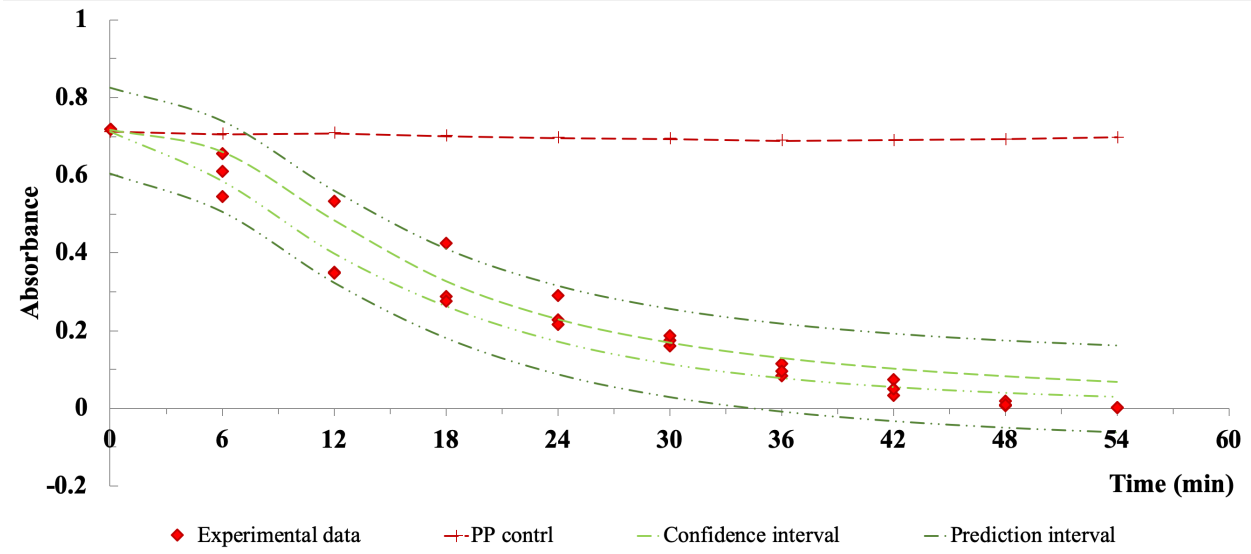
Boltzmann sigmoidal model

B. Trial 2

Figure C-1 (cont'd)



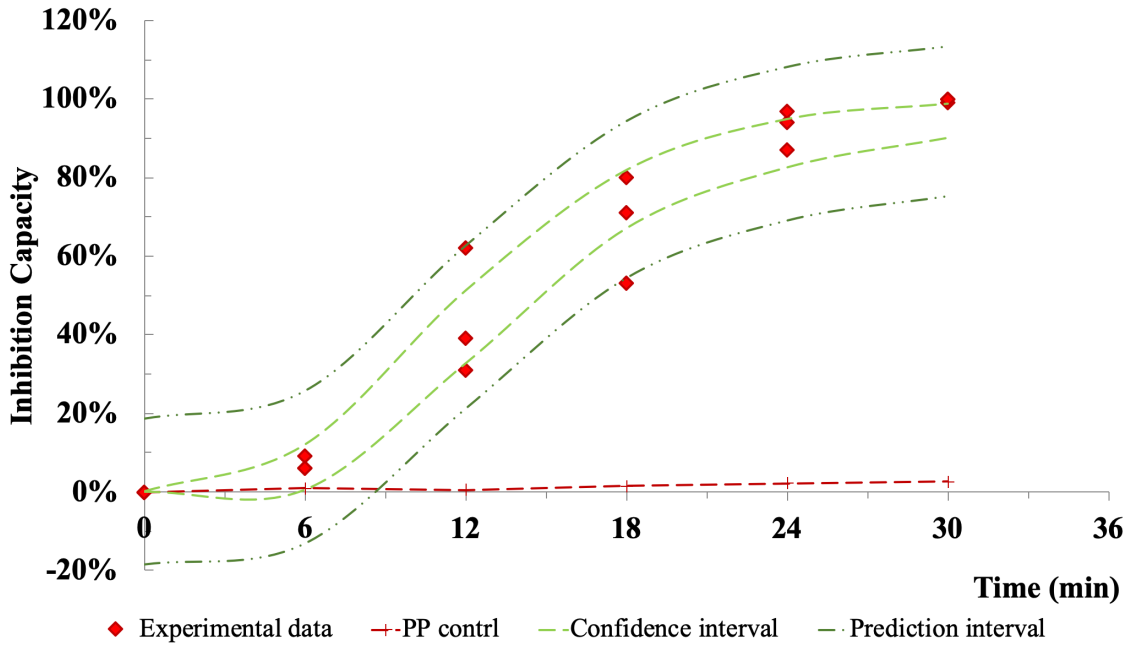
Dose-response model



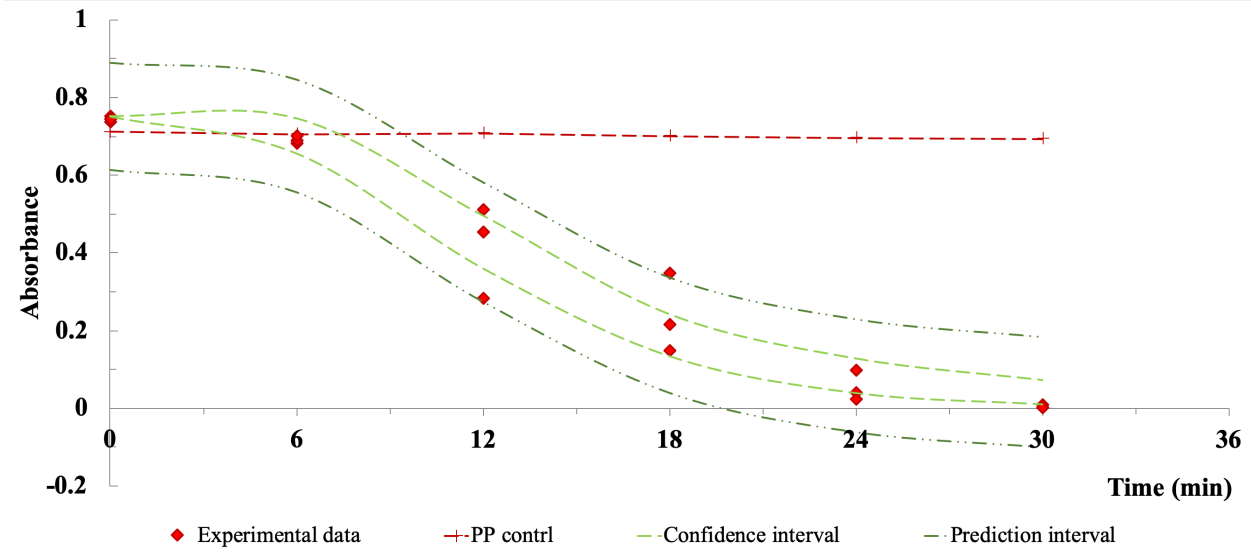
Boltzmann sigmoidal model

C. Trial 3

Figure C-1 (cont'd)



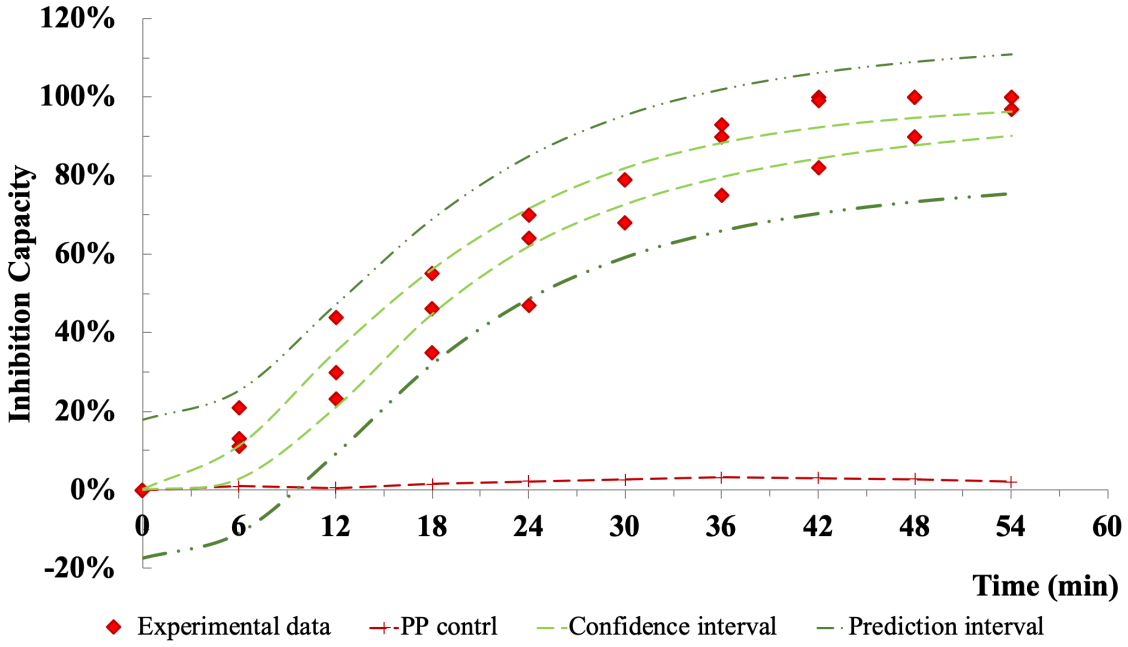
Dose-response model



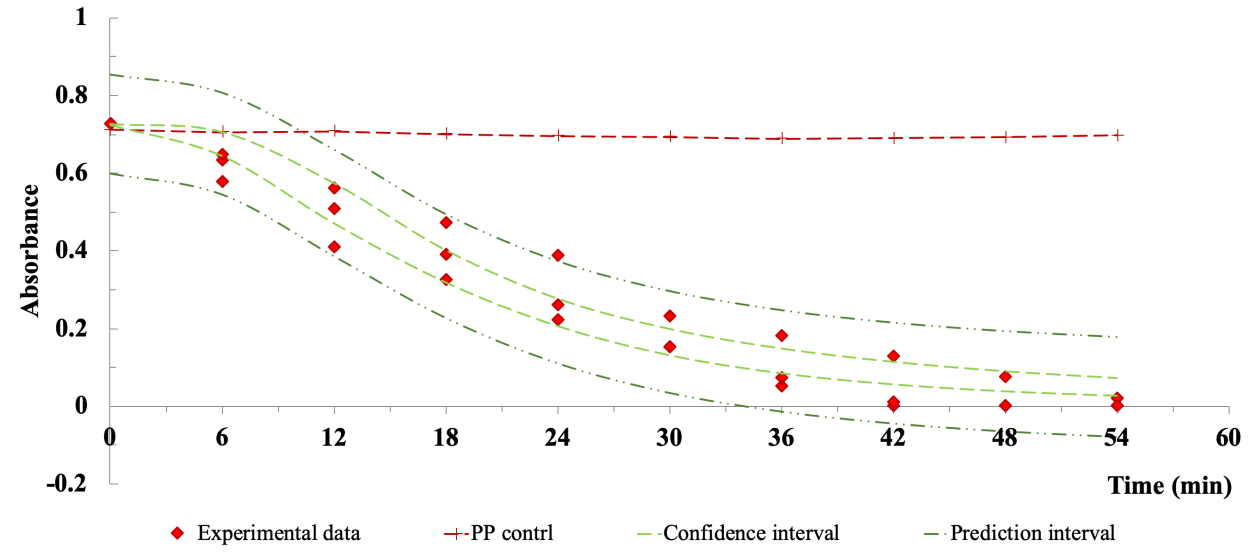
Boltzmann sigmoidal model

D. Trial 4

Figure C-1 (cont'd)



Dose-response model



Boltzmann sigmoidal model

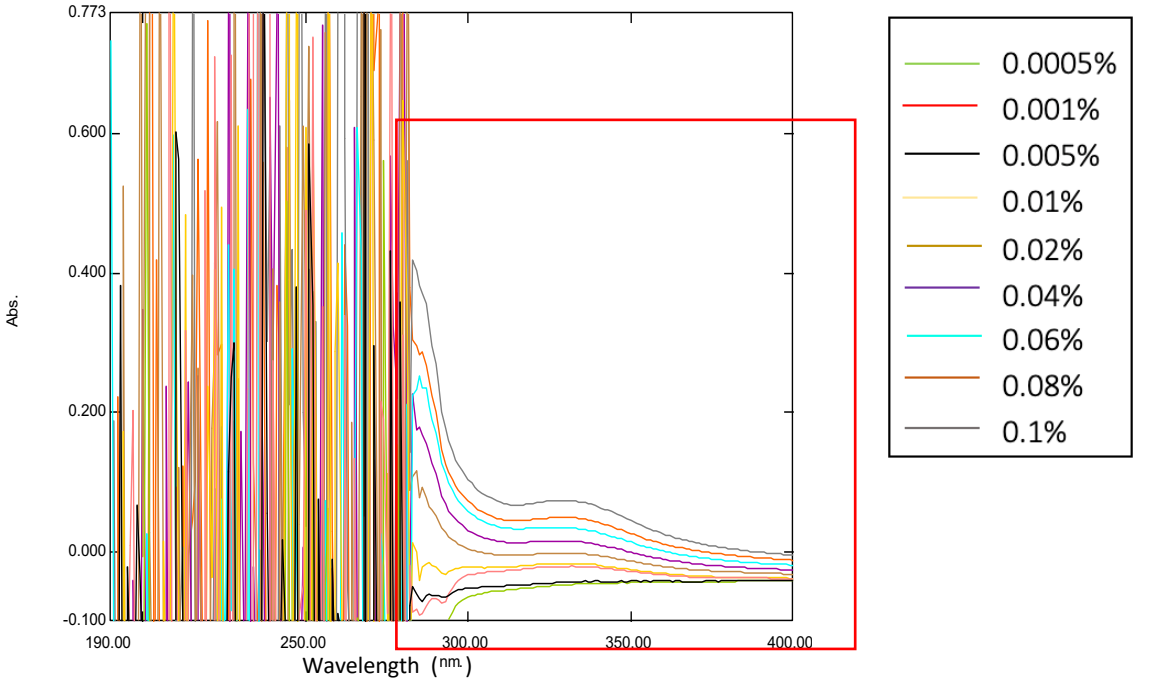
E. Trial 5

Table C-1 Fitted regression equations estimated by the dose-response and Boltzmann sigmoidal models for the antioxidative recoverability test.

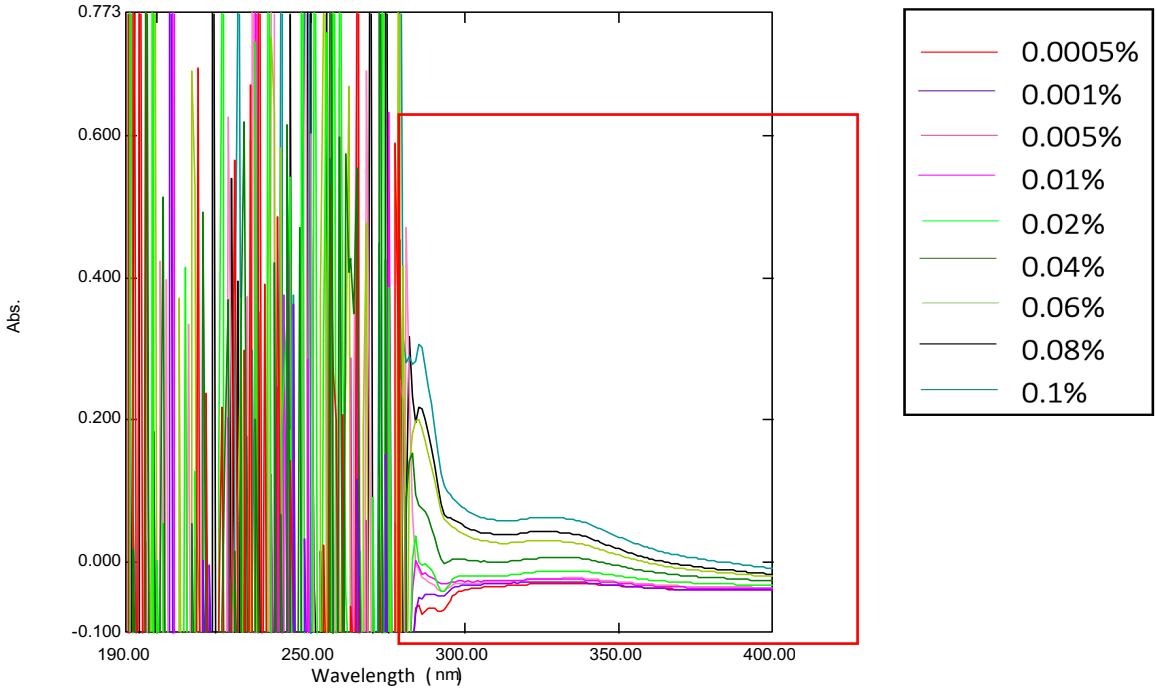
	Dose-response Model $\text{IC}\ (\%) = \frac{100}{1 + 10^{[(t_0 - t) \times \text{Slope}]}}$	**Boltzmann Sigmoidal Model** $y = \frac{-A'_2}{1 + exp^{(\frac{t - t_0}{\text{Slope}})}} + A'_2$
Trial 1	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.682 - t) \times 3.518]}}$	$y = \frac{-0.748}{1 + exp^{(\frac{t - 0.680}{-0.126})}} + 0.748$
Trial 2	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(0.939 - t) \times 1.893]}}$	$y = \frac{-0.736}{1 + exp^{(\frac{t - 0.936}{-0.230})}} + 0.736$
Trial 3	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(1.180 - t) \times 2.040]}}$	$y = \frac{-0.717}{1 + exp^{(\frac{t - 1.180}{-0.213})}} + 0.717$
Trial 4	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(1.120 - t) \times 3.443]}}$	$y = \frac{-0.751}{1 + exp^{(\frac{t - 1.115}{-0.128})}} + 0.751$
Trial 5	$\text{IC}\ (\%) = \frac{100}{1 + 10^{[(1.252 - t) \times 2.363]}}$	$y = \frac{-0.727}{1 + exp^{(\frac{t - 1.252}{-0.184})}} + 0.727$

Table C-2 Experimental data of the antioxidative recoverability test.

Trial	Sample	Absorbance									
		0 min	**6 min**	**12 min**	**18 min**	**24 min**	**30 min**	**36 min**	**42 min**	**48 min**	**54 min**
1	**1**	0.748	0.252	0.017	0.000	N/A	N/A	N/A	N/A	N/A	N/A
	2	0.745	0.155	0.000	0.000	N/A	N/A	N/A	N/A	N/A	N/A
	3	0.748	0.295	0.083	0.002	N/A	N/A	N/A	N/A	N/A	N/A
2	**1**	0.734	0.397	0.153	0.330	0.000	0.000	0.000	N/A	N/A	N/A
	2	0.735	0.504	0.293	0.152	0.043	0.004	0.000	N/A	N/A	N/A
	3	0.736	0.492	0.367	0.188	0.118	0.069	0.020	N/A	N/A	N/A
3	**1**	0.716	0.608	0.350	0.286	0.226	0.160	0.082	0.032	0.006	0.000
	2	0.717	0.543	0.347	0.275	0.215	0.174	0.114	0.072	0.018	0.000
	3	0.717	0.655	0.532	0.423	0.288	0.185	0.095	0.050	0.009	0.000
4	**1**	0.751	0.682	0.282	0.147	0.042	0.000	N/A	N/A	N/A	N/A
	2	0.736	0.689	0.511	0.346	0.098	0.007	N/A	N/A	N/A	N/A
	3	0.744	0.701	0.452	0.215	0.022	0.000	N/A	N/A	N/A	N/A
5	**1**	0.727	0.632	0.560	0.472	0.387	0.231	0.181	0.128	0.075	0.021
	2	0.726	0.646	0.508	0.391	0.261	0.152	0.052	0.000	0.000	0.000
	3	0.727	0.577	0.410	0.326	0.221	0.151	0.074	0.010	0.000	0.000
Negative Control		0.712	0.705	0.708	0.701	0.696	0.693	0.689	0.690	0.692	0.697

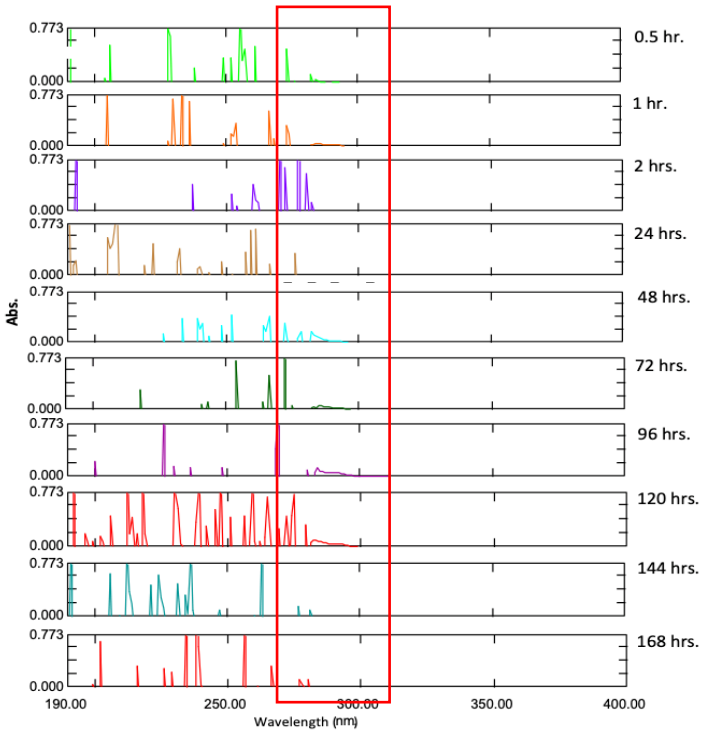


A. The crude phenolic extract in 50% ethanol

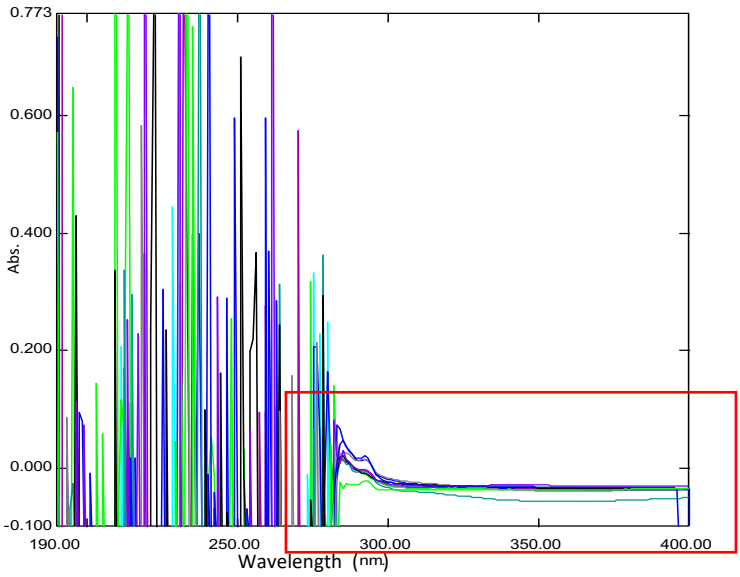


B. The crude phenolic extract in 10% ethanol

Figure C-2 Overlapped UV-Vis spectra of the crude phenolic extract from avocado peels in aqueous ethanol at various concentrations.



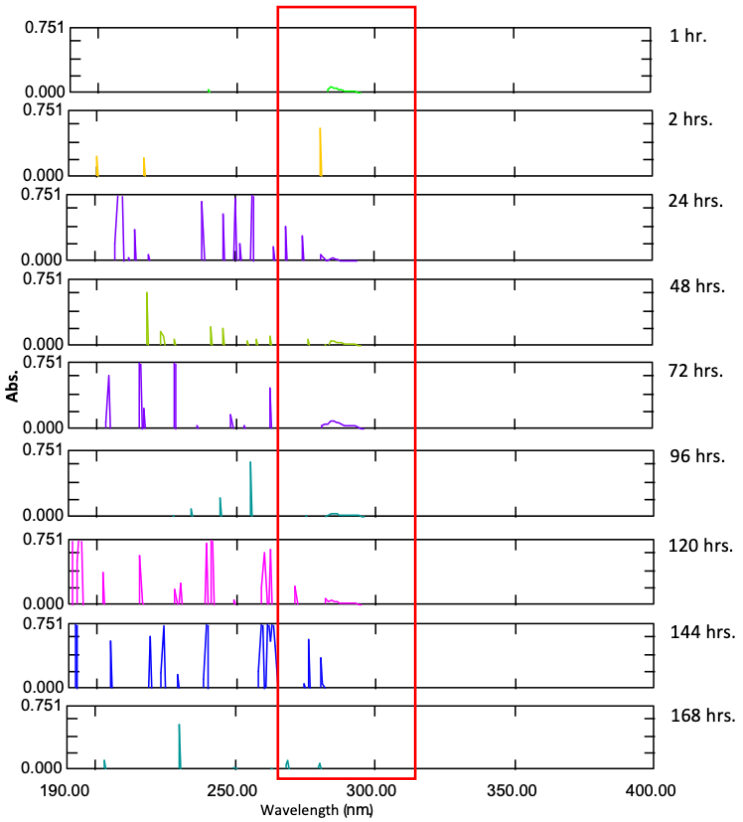
Sample 1



Sample 2

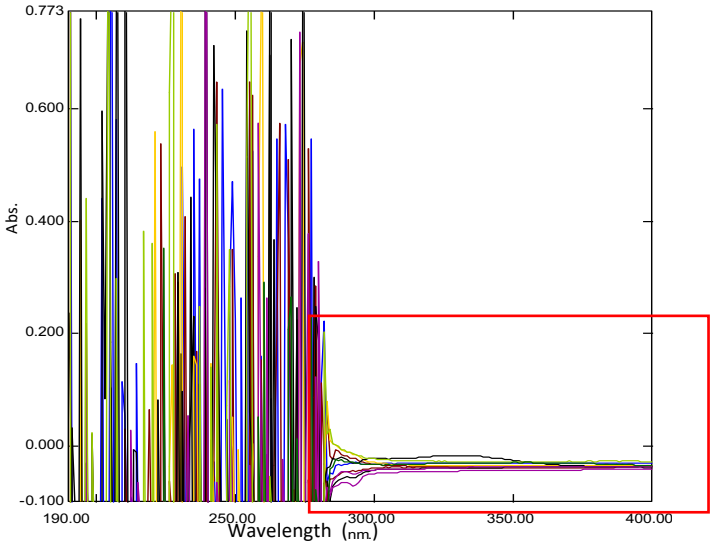
Figure C-3 UV-Vis spectra of phenolic compounds released from coated PP films in each migration cell.

Figure C-3 (cont'd)



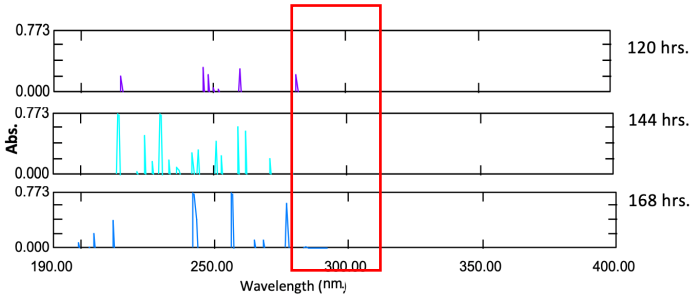
Sample 3

A. Coated PP films in 95% aqueous ethanol at 4°C

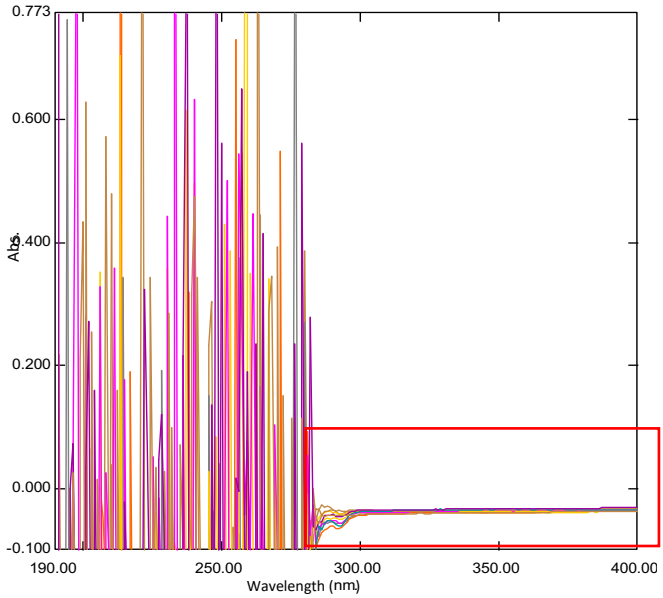


Sample 1

Figure C-3 (cont'd)



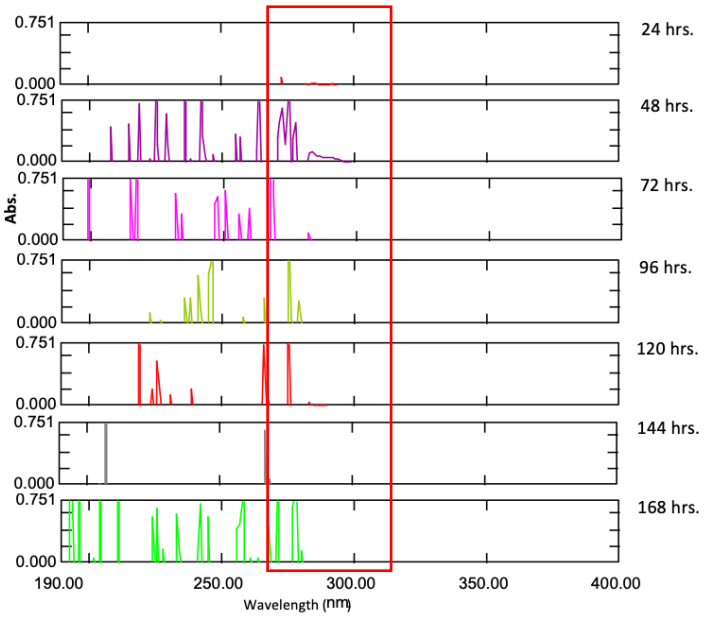
Sample 2



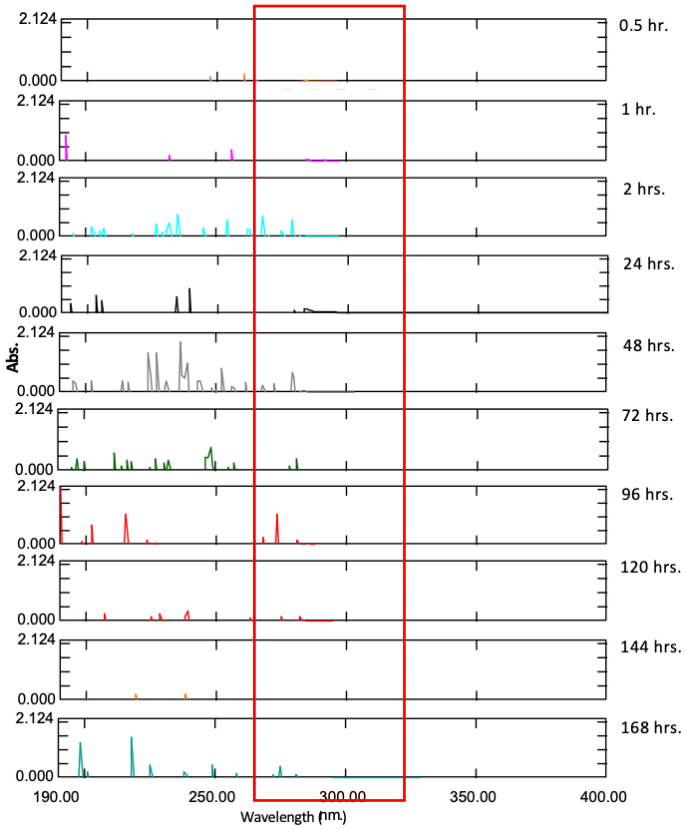
Sample 3

B. Coated PP films in 50% aqueous ethanol at 4°C

Figure C-3 (cont'd)

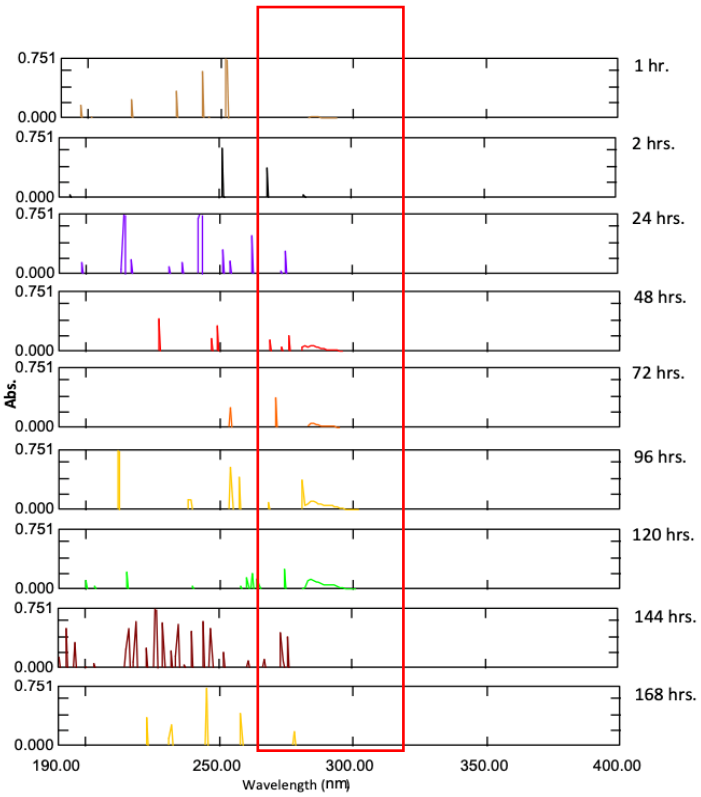


Sample 1



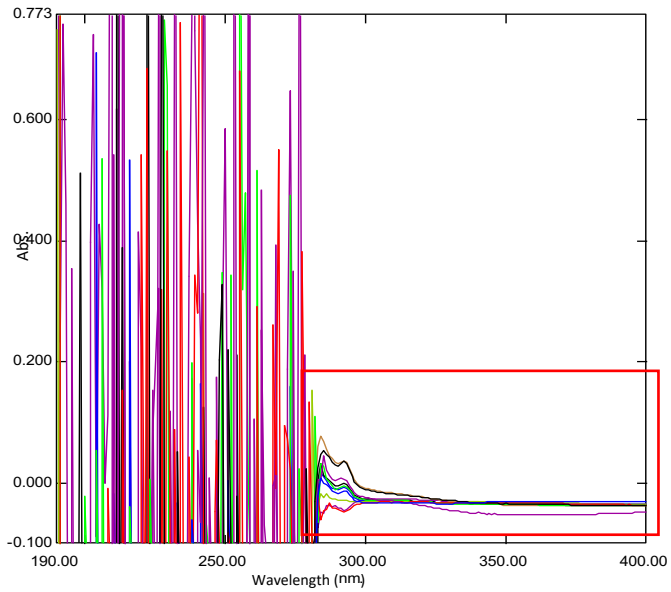
Sample 2

Figure C-3 (cont'd)



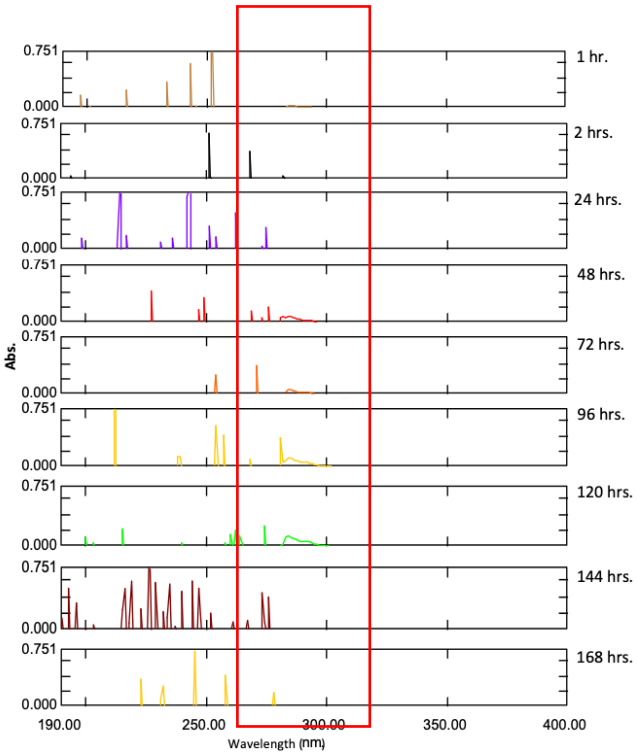
Sample 3

C. Coated PP films in 10% aqueous ethanol at 4°C

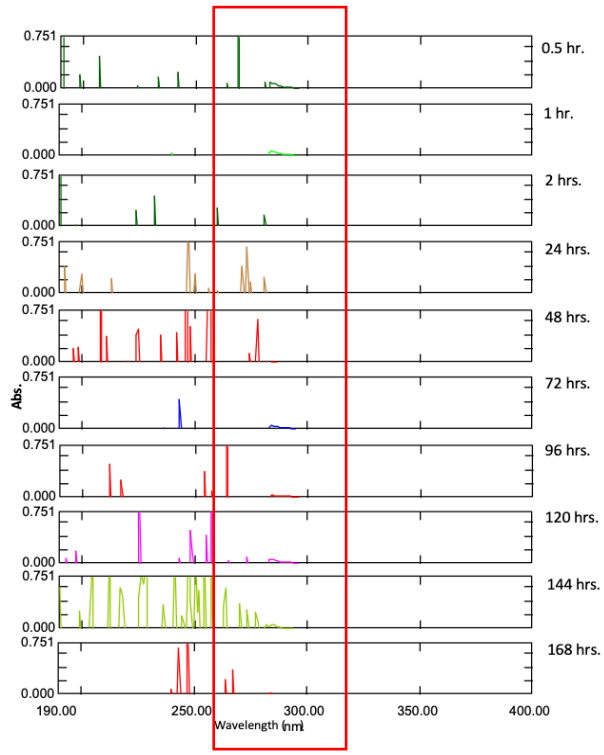


Sample 1

Figure C-3 (cont'd)



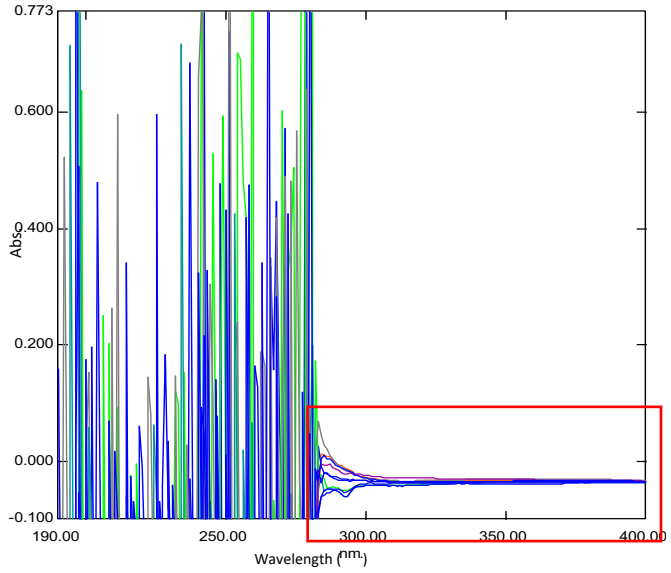
Sample 2



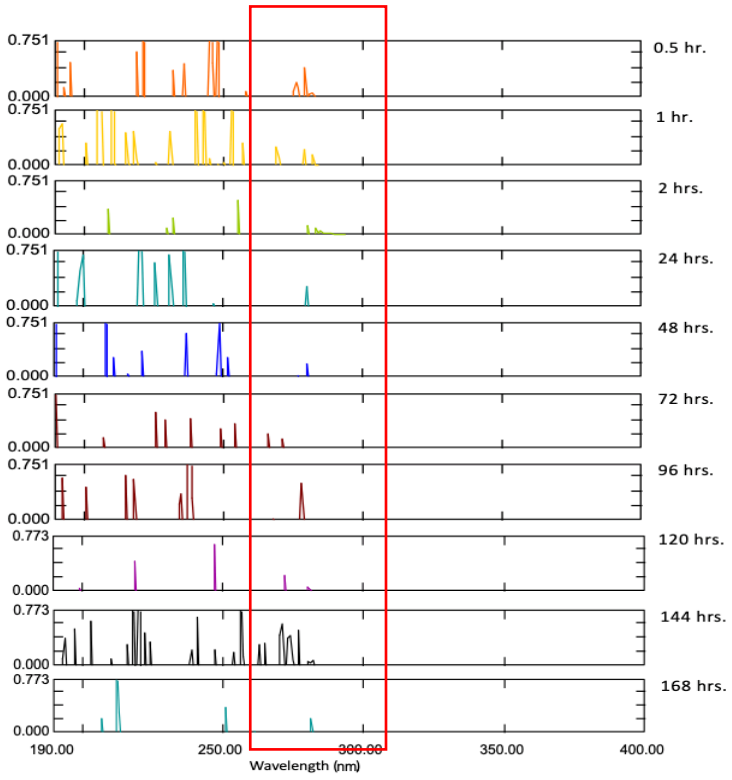
Sample 3

D. Coated PP films in 95% aqueous ethanol at 40°C

Figure C-3 (cont'd)

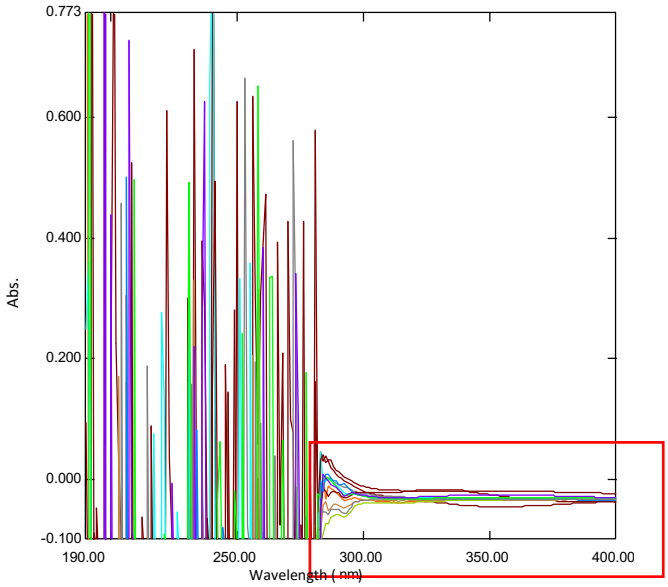


Sample 1



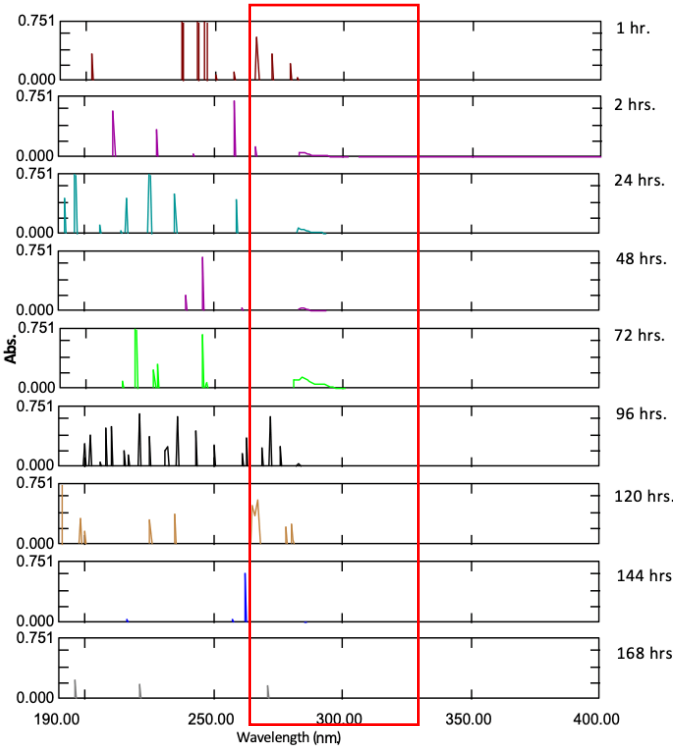
Sample 2

Figure C-3 (cont'd)



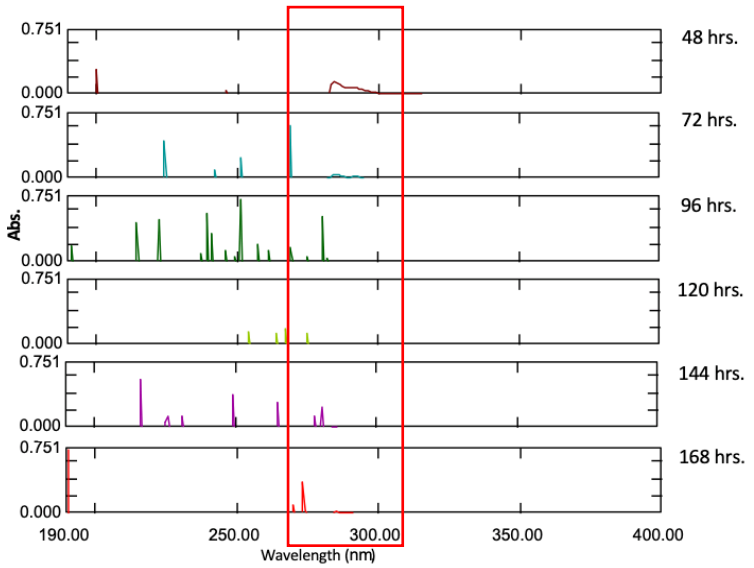
Sample 3

E. Coated PP films in 50% aqueous ethanol at 40°C

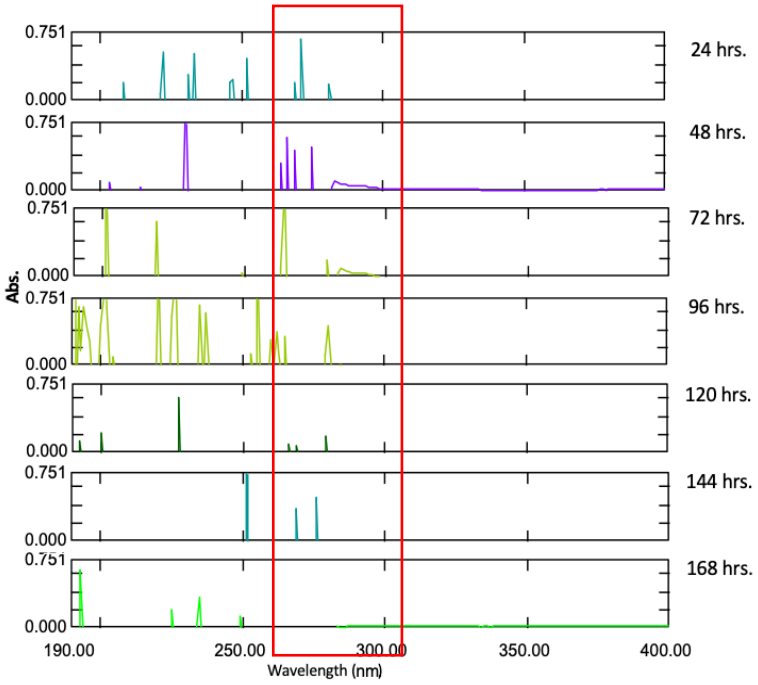


Sample 1

Figure C-3 (cont'd)



Sample 2



Sample 3

F. Coated PP films in 10% aqueous ethanol at 40°C

Table C-3 Phenolic compound size referenced for the mode of activity analysis of the antioxidant coating.

Compound Name	Size (nm)	References
Catechin	0.8×1.3	Ariga (2012)
Quercetin-3-O-rutinoside (rutin)	1.27×0.6	Yang et al. (2015)
Protocatechuic Acid	$0.9 \times 0.68 \times 0.29$	Barahuie et al. (2013)

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