



University of
Massachusetts
Amherst

Tocopherol regeneration by phospholipids in soybean oil-in-water emulsions: effect of tocopherol homologue and emulsifier type

Item Type	Thesis (Open Access)
Authors	Samdani, Gautam
DOI	10.7275/11423638
Download date	2025-05-13 12:57:26
Link to Item	https://hdl.handle.net/20.500.14394/33649

**Tocopherol regeneration by phospholipids in soybean oil-in-water emulsions: effect of
tocopherol homologue and emulsifier type**

A Thesis Presented

By

GAUTAM SAMDANI

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

February 2018

Department of Food Science

© Copyright by Gautam Samdani 2018

All Rights Reserved

**TOCOPHEROL REGENERATION BY PHOSPHOLIPIDS IN SOYBEAN OIL-IN-WATER EMULSIONS:
EFFECT OF TOCOPHEROL HOMOLOGUE AND EMULSIFIER TYPE**

A Thesis Presented

By

GAUTAM SAMDANI

Approved as to style and content by:

Eric A. Decker, Chair

David J. McClements, Member

Maria G. Corradini, Member

Eric A. Decker, Department Head
Department of Food Science

ACKNOWLEDGMENTS

I would like to thank Dr. Eric Decker for his guidance and support on this research and in my pursuit of a Master's degree. I would like to take this opportunity to thank Dr. Maria Corradini and Dr. David J. McClements for being a member of my committee and for their valued feedbacks.

Many thanks to all Decker lab members and all my friends for their support and friendship throughout the duration of my stay here. A big thank you to our lab manager Jean Alamed for all her help without which completion of my research would not have been possible in a timely manner.

Finally, I take this opportunity to thank my family for their unconditional love and support all my life. My Master's degree would not have been possible without the love and immense support of my parents, so a special thank you to them for being my pillars of strength and supporting me in all my endeavors. This one is for both of you!!

ABSTRACT

TOCOPHEROL REGENERATION BY PHOSPHOLIPIDS IN SOYBEAN OIL-IN-WATER EMULSIONS: EFFECT OF TOCOPHEROL HOMOLOGUE AND EMULSIFIER TYPE

FEBRUARY 2018

GAUTAM SAMDANI, B.TECH, INSITTUTE OF CHEMICAL TECHNOLOGY, MUMBAI

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Dr. Eric A. Decker

Phospholipids can regenerate oxidized tocopherols and help delay lipid oxidation. The impact of emulsifier type, tocopherol homologue and phospholipid head group on tocopherol-phospholipid interaction was investigated in this study.

Three μmol tocopherol/kg emulsion and 15.0 μmol /kg emulsion of PE or PS were dissolved in oil and emulsions were prepared. Tween 20 or bovine serum albumin(BSA) was used as emulsifier and the continuous phase contained 10mM imidazole/acetate buffer at pH 7. Lipid hydroperoxides and hexanal were measured as lipid oxidation products and the lag phase was determined. With Tween 20 as the emulsifier, α and δ -tocopherol had a hexanal lag phase of 2 and 4 days respectively. PE and PS both extended the lag phase to 7 and 10 days respectively in presence of δ -tocopherol. Whereas, PS extended the lag phase to 6 days and PE could not exhibit any synergism with α -tocopherol. With BSA as the emulsifier, α and δ -tocopherol had a lag phase of 4 days. PE and PS extended the lag phase to 11 days and 10 days respectively in presence of δ -tocopherol and to 7 and 8 days respectively in presence of α -tocopherol. PE and PS both exhibited synergism with mixed tocopherol and the extent of synergism was in less than δ -tocopherol but more than α -tocopherol.

Phospholipids could potentially be used with tocopherols to improve the oxidative stability of emulsions. PE was more effective with BSA whereas PS was equally effective with both emulsifiers.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS.....	iv
ABSTRACT.....	v
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER	
1. INTRODUCTION.....	1
2. LITERATURE REVIEW.....	3
2.1. Introduction.....	3
2.2. Mechanism of lipid oxidation in emulsions.....	4
2.3. Factors affecting lipid oxidation in emulsions.....	5
2.3.1. Droplet interfacial area.....	5
2.3.2. Droplet interface and its properties.....	5
2.3.3 Other factors.....	6
2.4. Antioxidants and their mechanism of actions.....	6
2.4.1. Metal chelators.....	7
2.4.2. Free radical scavengers.....	8
2.4.3. Oxygen scavengers/ quenchers.....	10
2.4.4 Synergistic antioxidant activity.....	11
2.5. Tocopherol.....	11
2.5.1. Tocopherol as antioxidant and pro-oxidant.....	14
2.6. Phospholipids.....	15
2.6.1. Effects of phospholipids on lipid oxidation.....	17
2.6.1.1. Metal chelation.....	17
2.6.1.2. Antioxidative effect due to Maillard browning products.....	18
2.6.1.3. Synergism with tocopherols.....	19
3. TOCOPHEROL REGENERATION BY PHOSPHOLIPIDS IN SOYBEAN OIL-IN-WATER EMULSIONS: EFFECT OF TOCOPHEROL HOMOLOGUE AND EMULSIFIER TYPE.....	20
3.1. Introduction.....	20
3.2. Materials and Methods.....	22
3.2.1 Preparation of Stripped Soybean Oil.....	23

3.2.2 Emulsion Preparation and Storage Conditions.....	23
3.2.3 Evaluation of Particle Size Distributions and Zeta Potential.....	24
3.2.4 Evaluation of Lipid Oxidation.....	24
3.2.5 Interaction Index.....	26
3.2.6 Statistical Analysis.....	26
4. RESULTS AND DISCUSSION.....	27
4.1. Droplet size and charge	27
4.2. Lipid Oxidation of 1% stripped soybean oil-in-water emulsions	28
4.2.1. Impact of PE and PS with or without tocopherols on the oxidation of Tween 20 stabilized SSO O/W emulsions	28
4.2.2. Impact of PE and PS with or without tocopherols on the oxidation of BSA stabilized SSO O/W emulsions.....	33
4.3. Summary of Interaction index	38
4.4. Summary of oxidation lag phases	38
4.5. Discussion	39
4.6. Conclusion.....	42
5. CONCLUSION AND FUTURE WORK	43
BIBLIOGRAPHY	44

LIST OF TABLES

Table	Page
2.1:	Tocopherol content (mg/100g of oil) in some common vegetable oils; adapted from (Shahidi & de Camargo, 2016) (tr- trace, nd- not detected) 13
2.2:	Phospholipid content in common foods. PL- total phospholipid content (g/kg total food); (PC, PE, PS PI content in g/kg total phospholipid); (Cui & Decker, 2016)..... 17
4.1:	Droplet size and zeta potential of 1% stripped soybean oil-in-water emulsions stabilized with either Tween 20 or bovine serum albumin (BSA) containing phosphatidylethanolamine (PE), phosphatidylserine (PS) and various tocopherols. Each value represents the mean (n=3) \pm standard deviations 28
4.2:	Interaction index between tocopherols and PE or PS in 1% stripped soybean oil-in-water emulsions stabilized with either Tween 20 or bovine serum albumin (BSA). 38
4.3:	Summary of hydroperoxide and hexanal lag phases in 1% SSO oil-in-water emulsion stabilized either with Tween 20 or BSA..... 39

LIST OF FIGURES

Figure	Page
2.1:	Proposed lipid oxidation mechanism in oil-in-water emulsion consisting of an oil phase, water phase and an interfacial region (usually covered with emulsifier) (Decker et al., 2017) 4
2.2:	Chemical structures of tocopherols and tocotrienols; adapted from (Shahidi & de Camargo, 2016) 12
2.3:	Reaction of α -tocopherol with lipid peroxy radical (R, R'= alkyl group); adapted from (Choe & Min, 2009) 14
2.4:	Structure of phospholipids; adapted from (Cui & Decker, 2016) 16
4.1:	Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with Tween 20 containing 3.0 μ mol/kg emulsion α -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS 29
4.2:	Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with Tween 20 containing 3.0 μ mol/kg emulsion δ -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS 31
4.3:	Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with Tween 20 containing 3.0 μ mol/kg emulsion mixed tocopherol and/or 15.0 μ mol/kg emulsion PE or PS..... 32
4.4:	Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with bovine serum albumin (BSA) containing 3.0 μ mol/kg emulsion α -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS..... 35
4.5:	Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with bovine serum albumin (BSA) containing 3.0 μ mol/kg emulsion δ -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS..... 36
4.6:	Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with bovine serum albumin (BSA) containing 3.0 μ mol/kg emulsion mixed tocopherol and/or 15.0 μ mol/kg emulsion PE or PS 38

CHAPTER 1

INTRODUCTION

Lipid oxidation can reduce shelf life, nutritional value of food and can produce toxic lipid oxidation products. Methods to prevent lipid oxidation are necessary for economical production of food and to benefit consumer health because it is recommended to use unsaturated fats in foods which are more prone to lipid oxidation than saturated fats.

Oil dispersions like emulsions are a big source of dietary fats and replacing the saturated fats with unsaturated ones in an emulsion increases the risk of rancidity. Currently, artificial antioxidants like BHT, BHA and EDTA are used commercially to prevent lipid oxidation. However, with increased consumer demand for elimination of synthetic additives has led researchers to look for other natural compounds that can help in preventing lipid oxidation. Unfortunately, natural antioxidants are often not as effective as their synthetic counterparts. Therefore, there is an increased need for research to find alternative natural antioxidant strategies and to improve the efficacy of the natural antioxidants currently known. Amino-group containing phospholipids can regenerate tocopherols back from the oxidized form in bulk oil and hence can improve the antioxidant activity of tocopherols. The ability of phospholipids to regenerate tocopherols in an emulsion system will be affected by the partitioning of individual compounds into the oil phase, interface and the aqueous phase. Partitioning can in turn be affected by various factors, some of which include the polarity of different tocopherol homologues and different phospholipid headgroups and by changing the emulsifier. A general literature review on lipid oxidation in emulsions, antioxidant mechanisms and the role of tocopherols and phospholipids as pro or antioxidants was also performed.

The effect of α , mixed and δ -tocopherol with and without phosphatidylethanolamine (PE) or phosphatidylserine (PS) on lipid oxidation in 1% stripped soybean oil-in-water emulsion at pH

7, stabilized with Tween 20 and stored at 20 °C was studied. The results suggested that the more polar tocopherol was more effective in delaying lipid oxidation by itself. PS showed synergism with all three tocopherols whereas PE showed synergism with mixed and δ -tocopherol only. The possible reason for PS to show synergism with α -tocopherol might be due to the greater surface affinity of PS as compared to PE.

A similar trend was observed when bovine serum albumin (BSA) was used as an emulsifier instead of Tween 20. α -tocopherol and PE exhibited an additive antioxidant effect whereas mixed and δ -tocopherol exhibited synergism with PE. PS exhibited synergism with all three tocopherols tested. δ -tocopherol again displayed strongest synergism amongst all tocopherols.

The reason for synergism between PE or PS and tocopherols is thought to be because of the ability of amino-group containing phospholipids to convert tocopherol quinone (oxidized form of tocopherol) back into tocopherol (active antioxidant). This helps in further delaying lipid oxidation.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Lipid oxidation is one of the reasons for food spoilage, food wastage and continues to be a challenge for the industry. With the desire to make food healthier and as per the dietary guidelines for Americans (DeSalvo, Olson, & Casavale, 2016), the industry is trying to replace saturated fats with polyunsaturated fats which increases the risk of rancidity. An ever-increasing demand for more natural and clean-label food makes it difficult to use synthetic antioxidants, hence there is need to find alternative antioxidant strategies utilizing natural compounds to delay lipid oxidation.

Food emulsions like mayonnaise, salad dressings, infant formula, beverages, creams, soups and sauces are some of the most common form of lipids (McClements, 2008) consumed in our diet apart from other sources like meat, cooking oil and baked goods like crackers. Each of the food system have their own challenges when it comes to effectively using antioxidants to delay lipid oxidation. Lipid oxidation can occur very rapidly in oil-in-water emulsions because of the large oil-water interface which increases the interaction between lipids and water soluble pro-oxidants like transition metal ions (iron, copper) and increases the accessibility of lipid droplets to the oxygen dissolved in aqueous phase. Transition metals like iron are one of major pro-oxidants which are found ubiquitously in foods, for example egg yolk contains 40 μ g iron/g. This explains why EDTA, a metal chelator, is a very potent antioxidant and is currently used in many food emulsions to delay lipid oxidation (Jacobsen et al., 2001). Fatty acid composition, concentration of antioxidants and pro-oxidants and location of antioxidant, aqueous phase pH and ionic

concentration, oxygen concentration, droplet size and interfacial properties can affect the rate of lipid oxidation in emulsions (Waraho, McClements, & Decker, 2011).

2.2. Mechanism of lipid oxidation in emulsions

Auto-oxidation of unsaturated fatty acid occurs in presence of reactive oxygen species and proceeds through three steps: initiation, propagation and termination. In O/W emulsions the reaction is accelerated by the presence of transition metal ions located in the aqueous phase as they can decompose the lipid peroxides into peroxy and alkoxy radicals which can further react with unsaturated lipids and propagate lipid oxidation. Lipid hydroperoxides are formed as primary products of lipid oxidation. Since hydroperoxides are unstable, secondary oxidation products like aldehydes, ketones, epoxides and polymers are formed because of the breakdown of hydroperoxides. Many of the secondary oxidation products are volatile and produce strong off-odors which negatively affects the sensory attributes of a product.

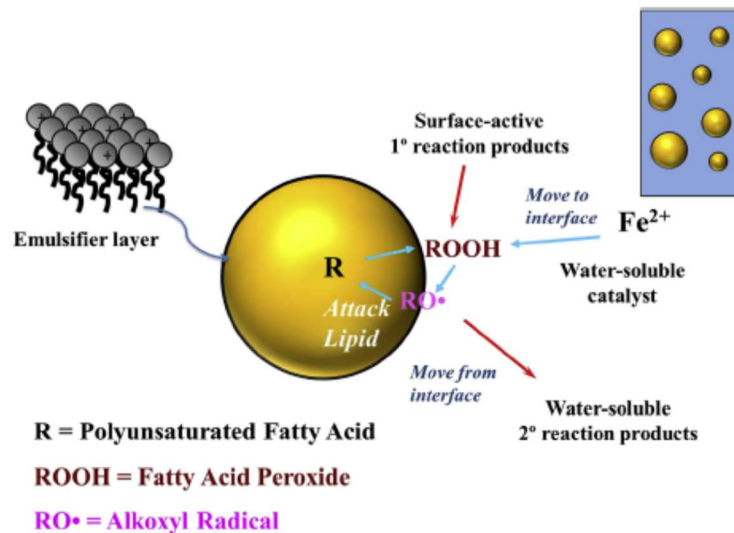


Figure 2.1: Proposed lipid oxidation mechanism in oil-in-water emulsion consisting of an oil phase, water phase and an interfacial region (usually covered with emulsifier)(Decker et al., 2017)

2.3. Factors affecting lipid oxidation in emulsions

Fatty acid composition, concentration of antioxidants and pro-oxidants and location of antioxidant, aqueous phase pH and ionic concentration, oxygen concentration, droplet size and interfacial properties can affect the rate of lipid oxidation in emulsions (Waraho et al., 2011).

2.3.1. Droplet interfacial area

Size of emulsion droplets in food can be really small, hence the surface area of a lipid droplet in an emulsion is much greater than that of bulk oil, droplets of size 100nm, 1 μ m, 10 μ m and 100 μ m will have a specific surface area of 65,220, 6,522, 652 and 65.2 m²/kg respectively (Decker et al., 2017). However, the droplet surface area alone might have very little impact on the rate of lipid oxidation indicating that most oil-in-water emulsions have extremely large surface area and the surface area cannot limit rate of oxidation reactions (Waraho et al., 2011).

2.3.2. Droplet interface and its properties

Chemical composition of the interfacial layer is very complex and contains not only the emulsifier but also surface-active materials like antioxidants, minor lipid components as well as mineral ions. Since the rate of lipid oxidation is often influenced by interactions between lipids and aqueous phase soluble pro-oxidants like metal ions, the interface and its properties can strongly influence lipid oxidation (Waraho et al., 2011). For example, emulsifiers with larger molecular dimensions can form a barrier and hence decrease the interactions between aqueous phase pro-oxidants and the lipid phase. Salmon oil-in-water emulsions prepared with Brij 700 as the emulsifier had lower rates of lipid oxidation as compared to the Brij 76 emulsions; whereas

increasing the emulsifier tail length slightly decreased the oxidation rate.(Chaiyasit, Silvestre, McClements, & Decker, 2000; Silvestre, Chaiyasit, Brannan, McClements, & Decker, 2000)

Droplet charge is determined by the type of emulsifier used (cationic, anionic or neutral) or the pH of the emulsion (Djordjevic, Cercaci, Alamed, McClements, & Decker, 2007; Hu, McClements, & Decker, 2003) which can affect the ability of the droplet to attract or repel cationic metals (Haahr & Jacobsen, 2008) and hence affect the rates of lipid oxidation.

2.3.3 Other factors

Oils contains a lot of minor compounds like phospholipids and free fatty acids which can influence lipid oxidation in emulsions (Chen, McClements, & Decker, 2011). Small molecule surfactants (Tweens) can form micelles in the aqueous phase above their critical micelle concentration. These micelles can alter the partitioning of compounds between the oil, interfacial and the aqueous regions and hence can affect lipid oxidation (Waraho et al., 2011). Addition of Tween 20 increased the antioxidant activity of δ and α -tocopherol in oil-in-water emulsions by solubilizing more tocopherol in the aqueous phase (Kiralan, Doğu-Baykut, Kittipongpittaya, McClements, & Decker, 2014).

2.4. Antioxidants and their mechanism of actions

Antioxidants are synthetic or naturally occurring substances that can delay the onset of oxidation or retard the rate of oxidation. The activity of antioxidants is strongly influenced by various factors, hence, compounds that are strong antioxidants in one system may not be very effective in other systems. Factors such as the extent of unsaturation in the lipid, the location of the antioxidant as influenced by its polarity, physical and chemical environments and interfacial

interactions can influence the antioxidant activity of a compound. Antioxidants can be classified as primary or secondary antioxidants depending on their mechanism of action.

Primary antioxidants can inhibit lipid oxidation as they can break the oxidation chain reaction by interfering at the propagation or initiation phase or in β -scission reactions by accepting free radicals and themselves form stable free radicals. Secondary antioxidants, also known as preventative antioxidants, decrease the rate of lipid oxidation by preventing the initiation of oxidation as these can react with pro-oxidants and inactivate them, however they do not convert free radicals into stable products. Metal chelators, oxygen scavengers/quenchers are examples of secondary antioxidants.

Antioxidants inhibit lipid oxidation through various mechanisms, some of the major mechanisms include: 1) metal chelators that can convert metal prooxidants into stable products, 2) singlet oxygen quenchers, 3) free radical scavengers which can inhibit free radical oxidation reactions and hence can stop oxidation at the initiation phase (preventative antioxidants) 4) chain breaking antioxidants that interrupt the propagation phase (proper antioxidants), and 5) synergists of proper antioxidants: these substance are not efficient antioxidants by themselves, but increases the activity of chain breaking antioxidants in a mixture (Pokorny, 2007).

2.4.1. Metal chelators

Metals can reduce the activation energy of lipid oxidation reaction and hence can catalyze the initiation reactions. Metal chelators are compounds that can bind to free metal ions and hence decrease the ability of metal ions to promote initiation reactions and the decomposition of hydroperoxides into secondary oxidation products like aldehydes (Pokorny, 2007). Chelators inhibit metal-catalyzed reactions by preventing metal redox cycling, formation of insoluble metal complexes, steric hindrance of metal-lipid interactions or oxidation intermediates (e.g.

hydroperoxides) and/or occupation of all metal coordination sites (Graf & Eaton, 1990). Conversely, under certain conditions, some chelators can increase metal solubility or alter the redox potential of metals thus increasing oxidative reactions. However, the antioxidative and/or prooxidative properties of metal chelators are often concentration dependent. It has been found that EDTA acts as a strong metal chelator at an EDTA:iron ratio of > 1 , whereas it can behave as a pro-oxidant at an EDTA:iron ratio of ≤ 1 . Similarly, there are many studies which report the antioxidant activity of phospholipids like PE, PC and PS due to metal chelation, whereas there are a few studies which find that phospholipids can accelerate lipid oxidation. This pro-oxidant/antioxidant activity of phospholipids might be due to different metal types and concentrations used in different studies (Cui & Decker, 2016). EDTA is one of the most effective metal chelators, along with citric, tartaric and phosphoric acids are just a few examples of compounds which can deactivate metals by forming stable coordination complexes with prooxidant metals, thus effectively inhibiting both metal-catalyzed initiation and decomposition of hydroperoxides.

2.4.2. Free radical scavengers

Free radical scavengers can inhibit lipid oxidation by interfering with chain initiation and/or propagation by donating a free hydrogen to the lipid radical, thereby stabilizing them (Choe & Min, 2005). Physical location (in oil phase or water phase or the interfacial region) and chemical reactivity determine the ability of a free radical scavenger to inhibit lipid oxidation. "The Polar Paradox" theory was thus an attempt to explain the importance of the physical location of an antioxidant in its activity (Porter, Black, & Drolet, 1989). It was proposed that non-polar antioxidants work best in a lipid dispersion such as oil-in-water emulsion whereas polar antioxidants work better in bulk oil. The explanation for greater efficacy of non-polar antioxidants

in lipid dispersions was attributed to the fact that non-polar antioxidants would reside in the oil droplet with the non-polar fatty acid, where they can scavenge the free radicals whereas polar antioxidants would partition to the aqueous phase in an emulsion where they cannot react with the non-polar fatty acid radicals. It was also stated that the non-polar antioxidant should be present at the interface where the oxidation is supposed to be happening (FRANKEL, HUANG, KANNER, & GERMAN, 1994; Porter et al., 1989). Waraho et al.(Waraho et al., 2011) discuss about various studies where non-polar antioxidants like α -tocopherol, carnosol, ascorbyl palmitate are more effective antioxidants than their polar counterparts like Trolox, carnosic acid and ascorbic acid in stripped oil emulsions, as predicted by the “polar paradox” theory; whereas there are other studies where the theory is not followed. A study was done where antioxidants were esterified with varying alkyl chain length to ensure that the compounds had similar free radical scavenging activity but had different polarities. It was found that antioxidant esters had maximum activity in the 8-12 carbon chain length, with longer and shorter being less effective. This was attributed to the fact that the shorter chain esterified antioxidants were more soluble in the aqueous phase, hence could not interact with lipid radicals whereas the longer chain esterified antioxidants were more soluble in the lipid droplet and less surface active, therefore were located in the interior of the lipid droplets (Laguerre et al., 2009; Laguerre et al., 2010). Finally, the safety and consumer acceptability of a free radical scavenger is important if it is to be used in a food product. Lot of strong free radical scavengers like BHT, TBHQ, PG are facing criticism from consumers due to their synthetic nature and due to the concerns of their potential negative health effects, hence there is a greater demand for natural free radical scavengers.

2.4.3. Oxygen scavengers/ quenchers

Oxygen is essential for degradation of lipids and can quickly interact with unsaturated fatty acids resulting in degradation of lipids. Singlet oxygen is the more reactive species which can promote lipid oxidation in foods containing photosensitizer whereas, triplet oxygen, the more common form takes part in the free radical chain reaction of lipid oxidation by quickly reacting with the alkyl radical and forming the peroxy radical. The peroxy radical then abstracts a hydrogen atom from an unsaturated lipid to form hydroperoxides producing a free radical on another fatty acid and this propagates the free radical chain reaction (Johnson & Decker, 2015). Use of quenching agents is one of the most effective way to reduce singlet oxygen oxidation besides packaging that excludes light. Quenching agents can inactivate singlet oxygen either by physical or chemical means. Physical quenching returns the singlet oxygen back into its ground state without generating oxidized products or consuming oxygen. Carotenoids like β -carotene, lycopene are a group of yellow to red compounds found naturally that can physically inactivate singlet oxygen. Carotenoids absorb the energy over the conjugated double bonds producing a ground state triplet oxygen and an excited-state carotenoid which vibrates and gives of the energy to the surrounding system generating heat (Min & Boff, 2002). Tocopherols are another class of compounds that can physically convert singlet oxygen to triplet oxygen by charge transfer and electron donation (KamalEldin & Appelqvist, 1996a). Both, tocopherols and carotenoids can quench singlet oxygen chemically which produces an oxidized antioxidant. However, for carotenoids, physical quenching is preferred as chemical inactivation degrades color (Min & Boff, 2002). Vacuum or modified atmosphere packaging can be used to decrease the oxygen levels in packaging which can help in improving lipid stability. However, packaging like polyethylene allows oxygen transmission other methods like ascorbate, iron or enzyme based oxygen scavengers can be used for complete removal of residual oxygen left in the system (Johnson & Decker, 2015).

2.4.4 Synergistic antioxidant activity

Synergistic antioxidant activity is a combination of two or more antioxidants which greater protection against lipid oxidation than the sum of the activities of individual compounds used separately. One possible combination could be that of a metal chelator and a free radical scavenger where the chelator would decrease the oxidation rates by inhibiting metal-catalyzed oxidation, hence fewer radicals would be generated in the system. This would make the free radical scavenger more effective as its concentration would be higher at any given time due to slower termination and auto-oxidation reactions. The other combination can be of two or more free radical scavengers where one reacts quickly with the free radical and is regenerated by the other antioxidant by radical transfer(Akoh, 2008).

2.5. Tocopherol

Tocopherols, also known as Vitamin E, are a group of compounds that possess strong antioxidant activity. The structures of different tocopherol and tocotrienol homologues are shown in Fig. 2. The structure is composed of a 6-chromanol ring with a 16-carbon side chain. Tocopherols have a saturated side chain whereas tocotrienols have 3 double bonds at carbons 3, 7 and 11. Tocopherols and tocotrienols exist as four homologues (alpha, beta, gamma and delta), which differ from each other by the location and number of methyl groups attached to the chromanol ring (Saini & Keum, 2016).

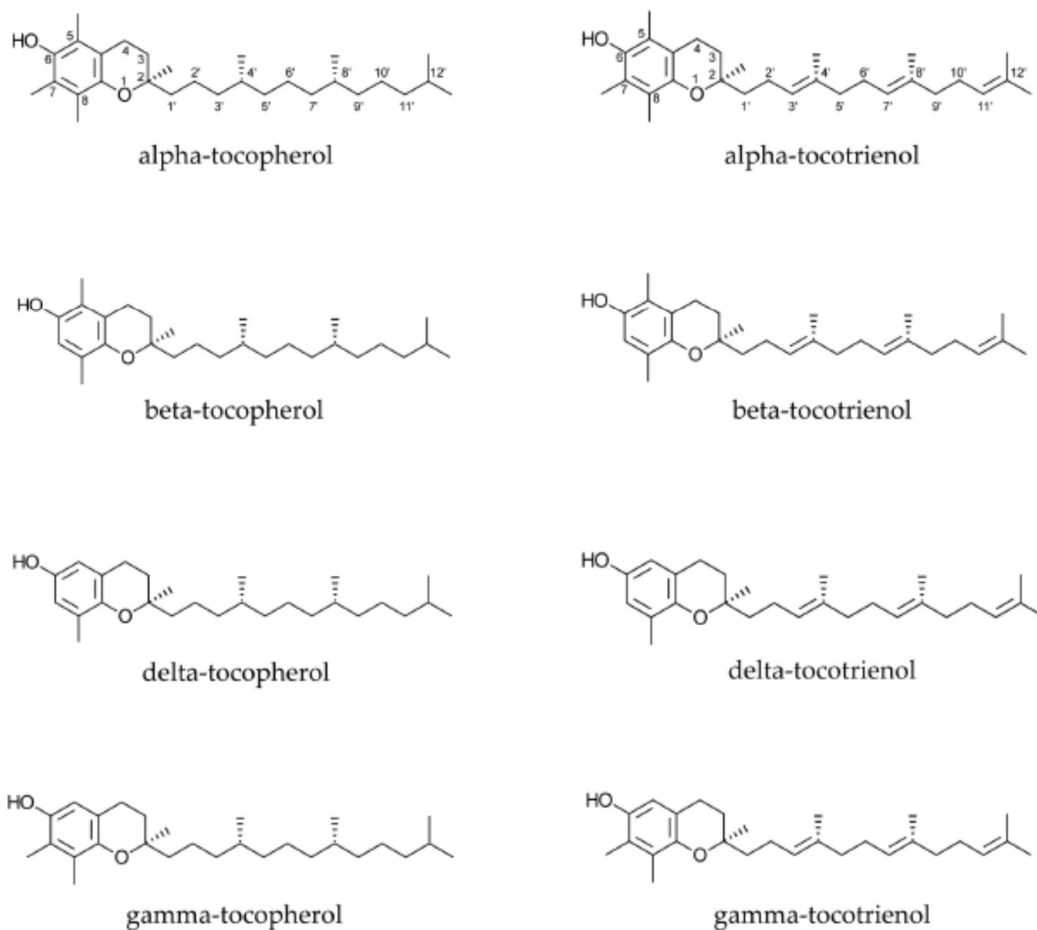


Figure 2.2: Chemical structures of tocopherols and tocotrienols; adapted from (Shahidi & de Camargo, 2016)

Tocopherols and tocotrienols are phenolic antioxidants found in most of the vegetable oils and hence help protecting the unsaturated fats from oxidation in oils. Table 1 gives a summary of tocopherol content in various vegetable oils. Hence, oilseeds and their lipid fractions serve as the major dietary source of Vitamin E, which is necessary for many important biological functions like prostaglandin synthesis, blood clotting and regulation of DNA synthesis.

OIL	APLHA-TOC	BETA-TOC	GAMMA-TOC	DELTA-TOC
BARLEY	14.2-20.1	0.6-1.9	3.5-15.1	0.9-4.6
COCONUT	0.2-1.82	tr-0.25	tr-0.12	nd-0.39
CORN	18.0-25.7	0.95-1.10	44.0-75.2	2.20-3.25
COTTONSEED	30.5-57.3	0.04-0.30	10.5-31.7	tr
LINSEED	0.54-1.20	nd-tr	52.0-57.3	0.75-0.95
OLIVE	11.9-17.0	nd-0.27	0.89-1.34	nd-tr
PALM	6.05-42.0	nd-0.42	tr-0.02	nd-0.02
PEANUT	8.86-30.4	nd-0.38	3.5-19.2	0.85-3.10
RAPESEED	18.9-24.0	nd-tr	37-51	0.98-1.90
RICE BRAN	0.73-15.9	0.19-2.5	0.26-8.00	0.03-2.70
SAFFLOWER	36.7-47.7	nd-1.20	tr-2.56	tr-0.65
SESAME	0.24-36.0	0.28-0.80	16.0-57.0	0.17-13.0
SOYBEAN	9.53-12.0	1.00-1.31	61.0-69.9	23.9-26.0
SUNFLOWER	32.7-59.0	tr-2.40	1.40-4.50	0.27-0.50
WHEATGERM	151-192	31.2-65.0	tr-52.3	nd-0.55

Table 2.1: Tocopherol content (mg/100g of oil) in some common vegetable oils ; adapted from (Shahidi & de Camargo, 2016)(tr- trace, nd- not detected)

Tocopherols can inhibit lipid oxidation by donating a phenolic hydrogen to peroxy radical, thereby converting it to a hydroperoxide. The oxidized tocopherol radical (tocopheroxyl radical) is stable and does not take part in the peroxidation process, rather it reacts with another peroxy radical to form a stable non-radical product (YAMAUCHI, YAGI, & KATO, 1996).

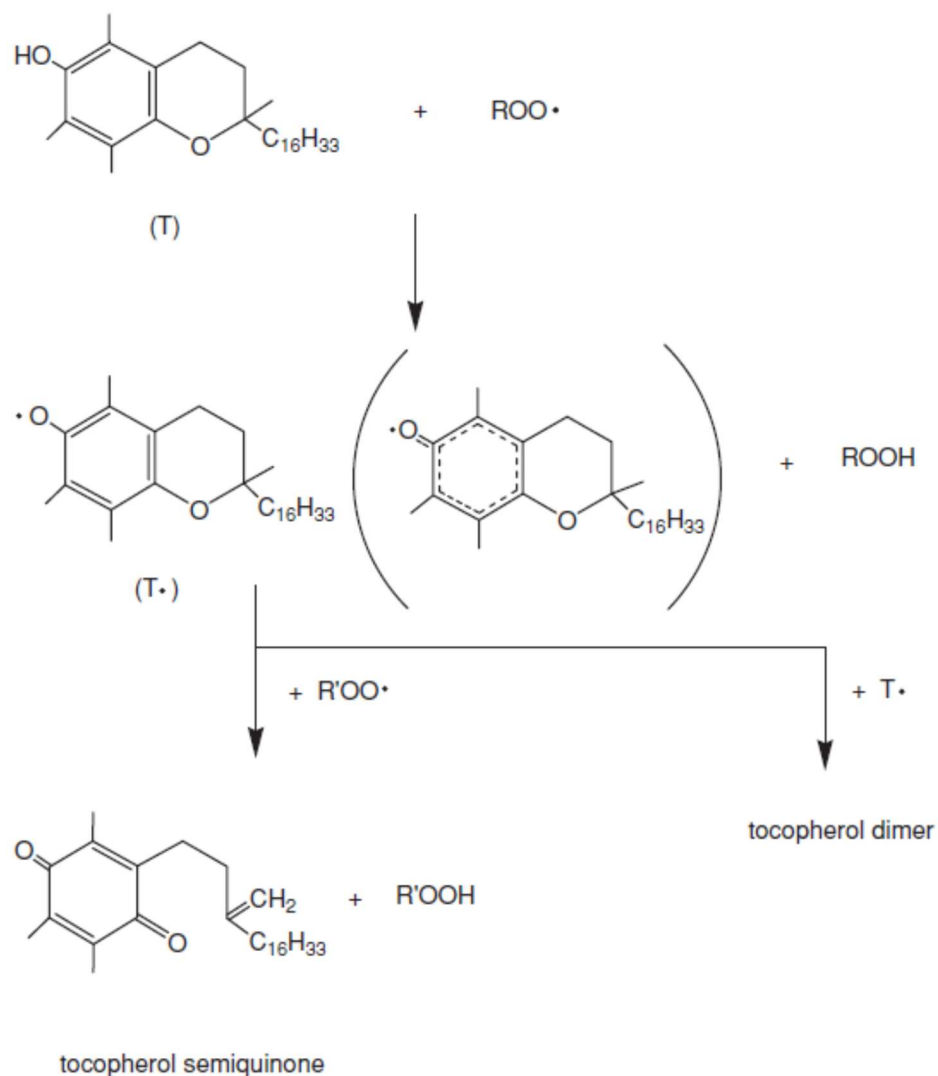


Figure 2.3: Reaction of α -tocopherol with lipid peroxy radical (R, R' = alkyl group); adapted from (Choe & Min, 2009)

2.5.1. Tocopherol as antioxidant and pro-oxidant

Although tocopherols have been reported to have strong antioxidant activity, there have been reports where α -tocopherol has shown to have some pro-oxidant activity. This pro-oxidant activity of α -tocopherol is thought to be due to α -tocopheroxy radicals, which at high concentrations can produce many undesirable side reactions, which can initiate chain reaction or enhance the rate of peroxidation (KamalEldin & Appelqvist, 1996b). The optimal concentration

of α -tocopherol for inhibition of hydroperoxides was found to be 100ppm in corn oil and 250-500ppm in 10% oil-in-water emulsion whereas the optimal concentration of γ -tocopherol in corn oil was 250-500ppm. α -tocopherol was found to be pro-oxidant at levels higher than 250ppm in corn oil and at levels above 500ppm in oil-in water emulsion; whereas γ -tocopherol had a pro-oxidant effect at 5000ppm in vacuum distilled corn oil. δ -tocopherol was found to be antioxidant at 2000ppm in both the systems (HUANG, FRANKEL, & GERMAN, 1994; HUANG, FRANKEL, & GERMAN, 1995). Wagner et. al found that γ and δ -tocopherol were more effective than α -tocopherol in absence and presence of a radical initiator (AIBN) in delaying lipid oxidation a 10% rapeseed oil triglyceride oil-in-water emulsion, they also found that α -tocopherol increased the formation of lipid hydroperoxides in presence and absence of a radical initiator and increased hexanal formation in presence of a radical initiator (Wagner, Isnardy, & Elmadfa, 2004). δ -tocopherol was found to have a stronger antioxidant effect than α -tocopherol in menhaden oil-in-water emulsion whereas α -tocopherol was more effective antioxidant in bulk menhaden oil (Chaiyasit, McClements, & Decker, 2005). This suggests that more polar tocopherols are stronger antioxidants in oil-in-water emulsions as they might be able to reside at the interface.

2.6. Phospholipids

Phospholipids are an integral part of biological membranes and hence are in all living species from which food is derived. A mixture of phospholipids, referred to as lecithin is commonly used in food because of desirable functional properties like anti-spattering, emulsification, non-stick releasing agent.

Structure of phospholipid consists of a glycerol backbone and a phosphate head group, which is typically found at the *sn*-3 position. The simplest phospholipid is phosphatidic acid (PA) which does not have any group attached to the phosphate and others are named after the group

attached to the phosphate group. For example, if the group attached to the phosphate group is choline, this phospholipid is called phosphatidylcholine (PC). Other substitution groups on the phosphate group include ethanolamine, serine and inositol, thus the phospholipids are named phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) respectively.

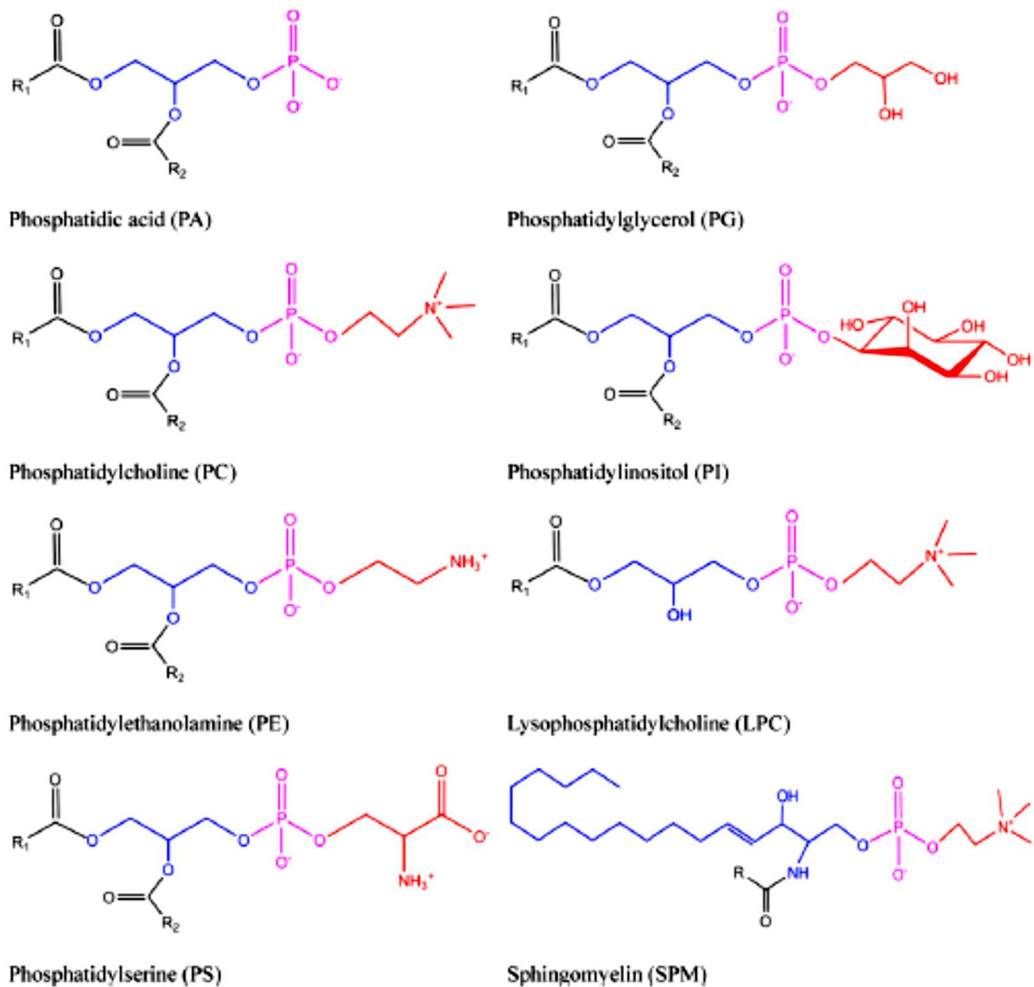


Figure 2.4: Structure of phospholipids; adapted from(Cui & Decker, 2016)

Many researchers have found that certain phospholipids can exert an antioxidant effect and can help in delaying lipid oxidation. Antioxidant activity of phospholipids could be due to one of the following mechanism, namely metal chelation, antioxidative effect of Maillard browning products or due to synergism with tocopherols.

FOOD	PL	PC	PE	PS	PI
SOYBEAN	20	450	263	50	141
CORN GERM	11	307	142	271	187
RAPSEED	15	487	83		184
PEANUT	6	435	81	40	242
LEMON JUICE	0.3	387	355	55	161
ORANGE JUICE	0.3	323	287	130	65
EGG LECITHIN		754	183		
SOYBEAN LECITHIN	754	183			

Table 2.2: Phospholipid content in common foods. PL- total phospholipid content (g/kg total food); (PC, PE, PS PI content in g/kg total phospholipid); (Cui & Decker, 2016)

2.6.1. Effects of phospholipids on lipid oxidation

Phospholipids can either be pro-oxidative or antioxidative and themselves can undergo oxidation. The antioxidant effect of phospholipids is attributed to one of 3 mechanisms which are discussed below.

2.6.1.1. Metal chelation

It was observed that the antioxidant activity of PC was possibly due to iron chelation in stripped soybean oil-in-water emulsion at pH 7 and the antioxidant activity of PC disappeared at pH 3, which is near the pK_a of PC as a result there is no charge at on the phosphate group. (Cardenia, Waraho, Rodriguez-Estrada, Julian McClements, & Decker, 2011). Yoon et al.(Yoon, 1987) found that phospholipids acted as antioxidants in bulk oil system when 1ppm of ferrous iron was added. PA significantly reduced iron-induced oxidation of arachidonic acid present in PC

liposomes (Viani, Cervato, Fiorilli, Rigamonti, & Cestaro, 1990). PA and PS could inhibit iron-induced oxidation of sardine oil in an oil-in water emulsion system (Dacaranhe & Terao, 2001). Although, the ability of phospholipids to bind iron does not always ensure that they can inhibit lipid oxidation as increased iron solubility can increase oxidation. Gal et al. (Gal, Pinchuk, & Lichtenberg, 2003) reported that increasing the ratio of PS or PA to PC in liposomes produced more negative charge and resulted in more copper being bound to the membrane and hence more lipid oxidation. PS, PC and PE increased the formation of TBA after exposure to hydroxyl radical (Brett & Rumsby, 1994).

2.6.1.2. Antioxidative effect due to Maillard browning products

It has been found previously that primary and secondary amines could inhibit lipid oxidation in stripped soybean oil whereas tertiary amines had no effect. The inhibitory effect was due to the formation of Maillard browning products formed by the reaction between octylamine (primary amine) and 4,5-epoxy-2-heptenal (lipid oxidation product). Similarly, PE contains a primary amine group which reacts with 4,5-epoxy-2-heptenal to produce antioxidative Maillard browning products (Alaiz, Zamora, & Hidalgo, 1996). The synergism between PC or PE and α -tocopherol in sardine oil could be due to Maillard reaction products measured at 430 nm. Maillard reaction in the presence of α -tocopherol was again confirmed through measurement of Strecker aldehydes, color changes and pyrrole content in a marine phospholipid liposome system and Maillard reaction was found to suppress the formation of volatile lipid oxidation (Bandarra, Campos, Batista, Nunes, & Empis, 1999; Shimajiri, Shiota, Hosokawa, & Miyashita, 2013). The antioxidant activity of amine-containing phospholipids (PC, PE and SPM) was due to the presence of α -tocopherol which was essential to produce antioxidative Maillard reaction products (Shimajiri et al., 2013).

2.6.1.3. Synergism with tocopherols

Previous studies have found that phospholipids can increase the antioxidant activity of tocopherol in bulk oil either by forming Maillard reaction products or by altering the physical location of tocopherols or by regenerating tocopherols. PE and PS regenerated α -tocopherol by reacting with α -tocopherol quinone and hence increased the antioxidant activity of tocopherol (Doert, Jaworska, Moersel, & Kroh, 2012). Antioxidant activity of phospholipids in perilla oil, specifically PE and PS was due to the presence of tocopherol whereas PC did not have a strong antioxidant activity with tocopherols (KASHIMA, CHA, ISODA, HIRANO, & MIYAZAWA, 1991). PC decreased and PE increased the antioxidant activity of tocopherol and Trolox in stripped soybean oil. When a combination of PC and PE was added, a higher PE: PC ratio was more effective in delaying lipid oxidation. It was also found that PE could regenerate α -tocopherol from α -tocopherol quinone in medium chain triglycerides (Cui, McClements, & Decker, 2015). Synergism was reported between PE, PC and cardiolipin and α -tocopherol, however the strongest synergism was exhibited by PE among the three (Bandarra et al., 1999).

CHAPTER 3

TOCOPHEROL REGENERATION BY PHOSPHOLIPIDS IN SOYBEAN OIL-IN-WATER EMULSIONS: EFFECT OF TOCOPHEROL HOMOLOGUE AND EMULSIFIER TYPE

3.1. Introduction

Lipid oxidation is one of the major causes affecting the quality and nutritive value of food. Oxidative deterioration of lipids produces volatile compounds that cause off flavors and oxidation of lipids can decrease nutritive value by accelerating the degradation of vitamins such as Vitamins C and E (FRANKEL, 1984; German, 1999). Traditionally, artificial antioxidants like 2,6-di-tert-butyl-p-hydroxytoluene (BHT), tert-butyl-4-hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) or synthetic metal chelators like ethylenediaminetetraaceticacid (EDTA) have been used to increase the shelf life of lipid containing products but there have been debates regarding deleterious health effects of these compounds (Martín et al., 2014; Nieva-Echevarría, Manzanos, Goicoechea, & Guillén, 2015). With an increased demand for cleaner food labels, there is a need to identify alternate antioxidant strategies with natural ingredients that inhibit lipid oxidation.

Tocopherols are natural antioxidants found abundantly in vegetable oil. The ability of tocopherols to be effective antioxidants in foods is dependent on several factors. Effectiveness is dependent on tocopherol homologue type with mixed tocopherols being more effective than α -tocopherol in foods such as margarines (Kanematsu et al., 1972) and pecan kernels (KING, 1986). In food oils, the antioxidant activity of tocopherols does not increase linearly as high concentrations will often have diminished activity (Choe & Min, 2009; Naumov & Vasilev, 2003). This means that food manufacturers can only add limited amounts of tocopherols to protect their products. Finally, tocopherols protect fatty acids by being preferentially oxidized and forming

antioxidant radicals that are not strong pro-oxidants (Choe & Min, 2009). This means that eventually, tocopherols are consumed and their ability to protect the food is lost.

The antioxidant activity of tocopherols could be increased when used in combination with other antioxidants. Combined antioxidants could be more effective because the antioxidant have different mechanisms and thus inhibit different oxidation pathways (e.g. free radical scavenger and chelator)⁷ or because antioxidants partition (Kiralan et al., 2014) into different place in the food (e.g. lipid vs water soluble free radical scavengers) and thus could inactive different radicals. In addition, the antioxidant activity of tocopherols can be improved by compounds that regenerate oxidized tocopherols (e.g. tocopherol quinone) to reform the active free radical scavenging state of the tocopherols. Synergistic activity of different compounds with endogenous tocopherol have been reported by several investigators. Melo et al.(Melo, Arrivetti, Leandro de Oliveira Rodrigues, Alencar, & Skibsted, 2016) reported synergism between α -tocopherol and acai seed/grape rachi seed extracts but this synergism was only observed at low tocopherol to plant extract ratios. Carlotti et al.(Carlotti et al., 1997) reported synergistic activity between certain amino acids and α -tocopherol at pH 5, but not at pH 7 in emulsions and micellar solutions.

Phospholipids form an essential part of all biological membranes and hence are found in practically all foods of both plant and animal origin. Phospholipids consists of a glycerol back bone with a phosphate group attached, generally at the sn-3 position(Cui & Decker, 2016). One of the strategies to enhance the activity of endogenous tocopherols includes the use of phosphatidylethanolamine (PE) or phosphatidylserine (PS), two types of phospholipid naturally found in oil that can regenerate oxidized tocopherols. Doert et al.(Doert et al., 2012) reported that PE and PS regenerated α -tocopherol by reacting with α -tocopherol quinone and hence increased the antioxidant activity of tocopherol. Kashima et al.(KASHIMA et al., 1991) found that the antioxidant activity of phospholipids in perilla oil, specifically PE and PS was due to the presence

of tocopherol whereas PC did not enhance the antioxidant activity of tocopherols. Cui et al.(Cui et al., 2015) reported that PC decreased and PE increased the antioxidant activity of α -tocopherol in bulk soybean oil. The observed increase in the antioxidant activity of α -tocopherol in presence of PE was due to the ability of PE to convert tocopherol quinone (oxidized tocopherol) back to the active form i.e. α -tocopherol. Bandarra et al.(Bandarra et al., 1999) reported that PE, PC and cardiolipin showed synergism with α -tocopherol, however the strongest synergism of the three was observed by PE.

While PE and PS have been shown to increase the antioxidant activity of tocopherols in bulk oil, no research has been conducted on their ability of PE and PS to enhance the antioxidant activity of tocopherols in oil-in-water emulsions. This work is important because while tocopherols and PS could easily interact in bulk oils, this might not be true in oil-in-water emulsions where the phospholipids and tocopherols could partition in different phases (e.g. emulsion droplet core, interface or aqueous phase) this inhibiting the ability of the phospholipids to interact with tocopherol quinones. Therefore, the objective of this study is to determine if PE and PS can enhance the antioxidant activity of tocopherols in oil-in-water emulsions and understand the effect of different tocopherol homologues and emulsifier types on the ability of PE and PS to act synergistically with tocopherols in delaying lipid oxidation

3.2. Materials and Methods

Soybean oil was purchased from a local store and stored at $-20\text{ }^{\circ}\text{C}$ until use. 1,2-Dioleoyl-sn-glycero-3- phosphoethanolamine (PE) and 1,2-Dioleoyl-sn-glycero-3- phosphor-L-serine sodium salt (PS) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Tween 20, bovine serum albumin (BSA), silicic acid, activated charcoal, α -tocopherol and δ -tocopherol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Mixed tocopherols, Decanox MTS-90G,

were donated by Archer Daniels Midland (Chicago, IL, USA). This mixture of tocopherols was 92.7% pure and consisted of 697 mg γ -tocopherol, 151 mg of β -tocopherol + δ tocopherol and 79mg α -tocopherol per gram. All other reagents were of HPLC grade or purer. Distilled and deionized water was used in all experiments. Glassware was soaked overnight in 2 N hydrochloric acid to remove metals, rinsed with distilled and deionized water and dried before use.

3.2.1 Preparation of Stripped Soybean Oil

In order to study tocopherol and phospholipid interactions without the interference of other oil components, a chromatographic column was used to separate soybean oil triacylglycerols from minor oil components like tocopherols, phospholipids, free fatty acids and mono and diacylglycerols according to the method reported by Cui et al.(Cui et al., 2015). Briefly, three layers were packed into a chromatographic column (3.0 cm internal diameter x 35 cm height). The bottom layer was packed with 22.5 g of silicic acid (washed with distilled and deionized water and activated at 110 °C for 24 h). Activated charcoal (5.6 g) was then used for the middle layer and another 22.5 g of silicic acid for the top layer. Commercial soybean oil (30 g) was mixed with 30 mL of hexane and the triacylglycerol fraction was eluted from the column using 270 ml of hexane. The solvent was then removed by a vacuum rotary evaporator (Rotavapor R 110, Buchi, Flawil, Switzerland) at 25°C, and the remaining solvent was evaporated by nitrogen flushing. Stripped soybean oil was stored at -80°C in the dark until emulsions were prepared.

3.2.2 Emulsion Preparation and Storage Conditions

Oil-in-water (O/W) emulsions were prepared using 1.0 wt% stripped soybean oil and 10 mM imidazole-acetate buffer, pH 7. Tween 20 or BSA was used as the emulsifier at a 1:10 emulsifier:oil ratio. The emulsion was prepared by adding phospholipids (15 μ mole/kg of emulsion) dissolved in chloroform \pm tocopherols (3 μ mole/kg of emulsion) dissolved in methanol

into a beaker and flushing with nitrogen gas to remove the solvents. Stripped soybean oil was then added to the beaker and stirred at room temperature for 30 min. Tween 20 and imidazole-acetate buffer were mixed in a separate beaker and then added to the beaker containing stripped soybean oil and a coarse emulsion was made by blending with a hand-held homogenizer (M133/1281-0, Biospec Products Inc., Bartlesville, OK) for 2 mins. The coarse emulsion was then homogenized with a microfluidizer (Microfluidics, Newton, MA, USA) at a pressure of 9 kpsi for three passes. During homogenization, ice was used to cover the homogenizer chamber and coil, to keep the emulsion cold. One milliliter of each emulsion was transferred into 10 mL GC vials (Supelco, Bellefonte, PA), capped with aluminum lids having PTFE/silicone septa and stored in the dark at 20°C.

3.2.3 Evaluation of Particle Size Distributions and Zeta Potential

Samples for droplet size distribution and zeta potential measurements were prepared by diluting the emulsion 10 times into 10 mM imidazole-acetate buffer, pH 7.0. Both particle size distributions and zeta potential of the emulsions were analyzed in a ZetaSizer Nano-ZS (Malvern Instruments, Worcestershire, UK)(Johnson, Tian, Roman, Decker, & Goddard, 2015). The particle size and zeta potential were determined right after emulsion preparation and at the end of each experiment. Each measurement was repeated thrice at room temperature.

3.2.4 Evaluation of Lipid Oxidation

Formation of primary and secondary (lipid hydroperoxides and hexanal, respectively) lipid oxidation products were quantified to determine the oxidative stability of the O/W emulsions. Lipid hydroperoxides were quantified using a modified version of the method reported by Shantha and Decker (SHANTHA & DECKER, 1994). Emulsions (0.3 mL) were vortexed three times (10 s each) with 1.5 mL of an isooctane:isopropanol (3:1, v/v) solution. The samples were then centrifuged

for 2 min at 3,000 rpm (Centrifric TM Centrifuge, Fisher Scientific, Fairlawn, NJ) after which 0.2 mL of the upper organic layer was mixed with 2.8 mL of methanol:butanol solution (2:1, v/v), 15 μ L of 3.94 M ammonium thiocyanate and 15 μ L of a ferrous iron solution. The ferrous iron solution was prepared by mixing 0.13 M BaCl_2 and 0.14 M FeSO_4 . Twenty min after iron addition, the absorbance of the samples was measured at 510 nm, using a Genesys 20 spectrophotometer (Thermo-Spectronic, Waltham, MA). Hydroperoxide levels were quantified from a cumene hydroperoxide standard calibration curve.

Headspace hexanal was quantified using a method described by Cardenia et al (Cardenia et al., 2011) using solid-phase microextraction-head space gas chromatography with flame ionization detection (SPME-GC-FID). The gas chromatograph was a Shimadzu GC-2014 (Shimadzu, Kyoto, Japan) equipped with an AOC-5000 autosampler (Shimadzu) and a split-splitless injector. An Equity DB-1 column (30 m x 0.32 mm x 1 mm film thickness, Supelco, Bellefonte, PA), was used for separation of volatiles. Samples were shaken and heated at 55°C for 10 min in an autosampler heating block before injection. A 50/30 mm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber needle (Supelco, Bellefonte, PA) was introduced into the vial for 2 min to absorb volatiles and then was transferred to the injector port to allow volatile desorption for 3 min at 250°C. Oven temperature was 65°C and run time was 6 min. The injector and detector temperatures were both set at 250°C. Helium was used as carrier gas at a flow rate of 1.0 mL/min with a split ratio of 1:7. Hexanal concentrations were determined from peak areas using a calibration curve prepared with hexanal standard solutions. Both lipid hydroperoxides and headspace hexanal were determined on the day of emulsion preparation (day 0) and then every 24 hours.

3.2.5 Interaction Index

To find out the interaction between antioxidants, interaction index was calculated as :

$$\frac{\{(\text{observed lag phase of combination} - \text{lag phase of control}) / [(\text{lag phase of tocopherol alone} - \text{lag phase of control}) + (\text{lag phase of phospholipid alone} - \text{lag phase of control})]\}}$$

An interaction index value of > 1 indicates a synergistic interaction between the antioxidants, a value =1 indicates additive effect and a value < 1 indicates antagonistic interaction between the antioxidants.(Kittipongpittaya, Panya, Phonsatta, & Decker, 2016; Melo et al., 2016; Peyrat-Maillard, Cuvelier, & Berset, 2003)

3.2.6 Statistical Analysis

Results are presented as means and standard deviations of analyses conducted in triplicate. Oxidation lag phases were defined as the first data point statistically greater than day zero within each treatment tested using one-way analysis of variance (ANOVA) with comparison of the oxidation measurement means performed using Tukey's HSD post hoc test ($p = 0.05$). Calculations were performed using Minitab version 18 (State College, PA, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Droplet size and charge

Table 4.1 shows the droplet size and charge for all emulsions studied. Overall, droplet size did not vary among treatments. In the Tween 20 emulsions, PS containing emulsions tended to be more negative than the rest of the emulsions. Emulsion droplet size did not increase during the entire storage studies.

sample	Tween 20		BSA	
	droplet size (nm)	zeta Potential (mV)	droplet size (nm)	Zeta Potential (mV)
control	210.7 ± 7.2 a	-8.42 ± 0.39 a	223.9 ± 11.1 a	-33.3 ± 1.2 a
PE	202.7 ± 2.0 a	-8.50 ± 0.58 a	209.5 ± 9.3 a	-33.3 ± 0.3 a
PS	208.2 ± 1.7 a	-12.27 ± 0.61 b	235.0 ± 3.0 a	-33.8 ± 0.8 a
α-tocopherol	203.8 ± 3.4 a	-7.74 ± 0.38 a	212.8 ± 4.6 a	-32.9 ± 0.9 a
δ-tocopherol	222.0 ± 1.3 a	-8.25 ± 0.06 a	233.4 ± 10.6 a	-33.5 ± 0.9 a
mixed tocopherol	203.9 ± 1.2 a	-8.50 ± 0.58 a	224.1 ± 16.0 a	-36.0 ± 1.0 a
α-tocopherol+ PE	197.3 ± 1.1 a	-7.69 ± 0.31 a	207.8 ± 4.5 a	-32.8 ± 0.1 a
α-tocopherol+ PS	200.3 ± 3.7 a	-9.93 ± 0.18 c	206.7 ± 5.3 a	-34.0 ± 0.8 a
δ-tocopherol+ PE	222.0 ± 2.5 a	-8.79 ± 0.22 a	235.2 ± 8.6 a	-33.0 ± 0.4 a
δ-tocopherol+ PS	225.6 ± 3.5 a	-11.01 ± 1.10 ab	234.9 ± 8.6 a	-34.7 ± 0.5 a
mixed tocopherol+ PE	204.0 ± 1.0 a	-9.40 ± 1.41 a	221.0 ± 2.4 a	-34.1 ± 0.5 a
mixed tocopherol+ PS	211.8 ± 3.8 a	-10.60 ± 0.63 a	219.7 ± 9.1 a	-36.2 ± 0.7 a

Table 4.1: Droplet size and zeta potential of 1% stripped soybean oil-in-water emulsions stabilized with either Tween 20 or bovine serum albumin (BSA) containing phosphatidylethanolamine (PE), phosphatidylserine (PS) and various tocopherols. Each value represents the mean (n=3) ± standard deviations

4.2. Lipid Oxidation of 1% stripped soybean oil-in-water emulsions

4.2.1. Impact of PE and PS with or without tocopherols on the oxidation of Tween 20 stabilized SSO O/W emulsions

The Tween 20-stabilized SSO emulsion had a lag phase for lipid hydroperoxides formation of 0 days and the lag phase of hexanal formation was 1 day. Addition of 3.0 µmol α-tocopherol/kg emulsion extended the hydroperoxide lag phase to 1 day and hexanal lag phase to 2 days. PE and PS did not affect the hydroperoxide and hexanal lag phase by themselves as compared to control. When PE was added with α-tocopherol in the emulsion it resulted in hydroperoxide lag phase of 1 day and hexanal lag phase was 2 days, indicating that PE had little to no impact on the antioxidant activity of α-tocopherol. However, the combination of PS and α-tocopherol extended hydroperoxide lag phase to 5 days and hexanal lag phases to 6 days. The interaction index for PS and α-tocopherol was 5 for both hydroperoxides and hexanal indicating synergism.

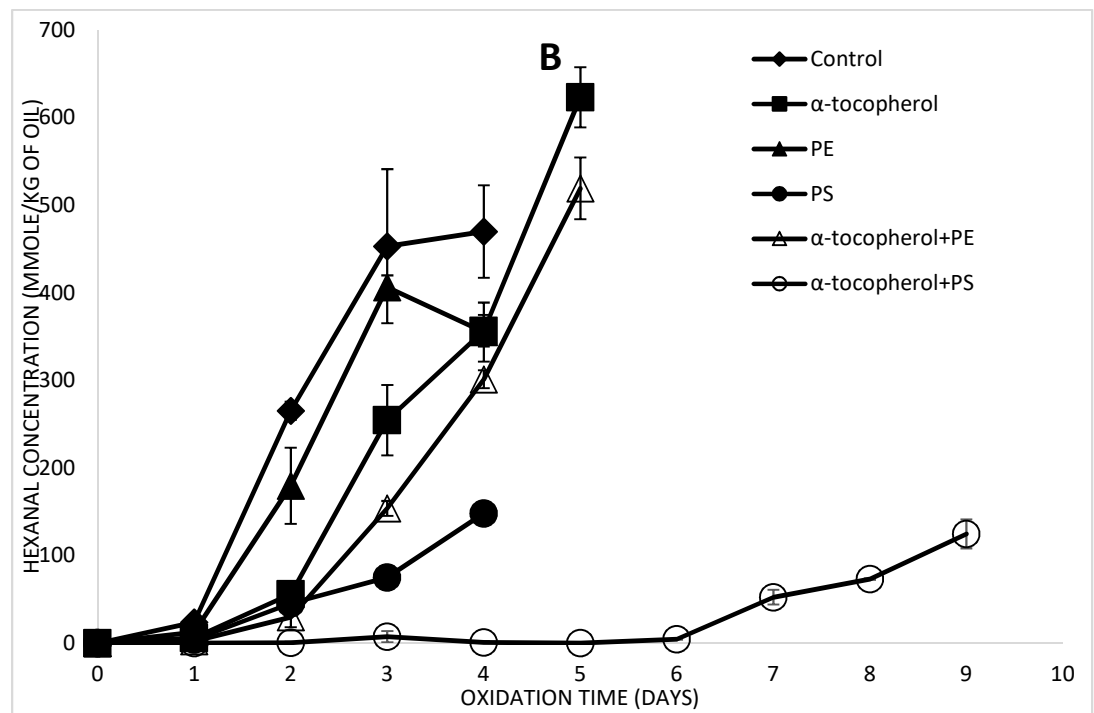
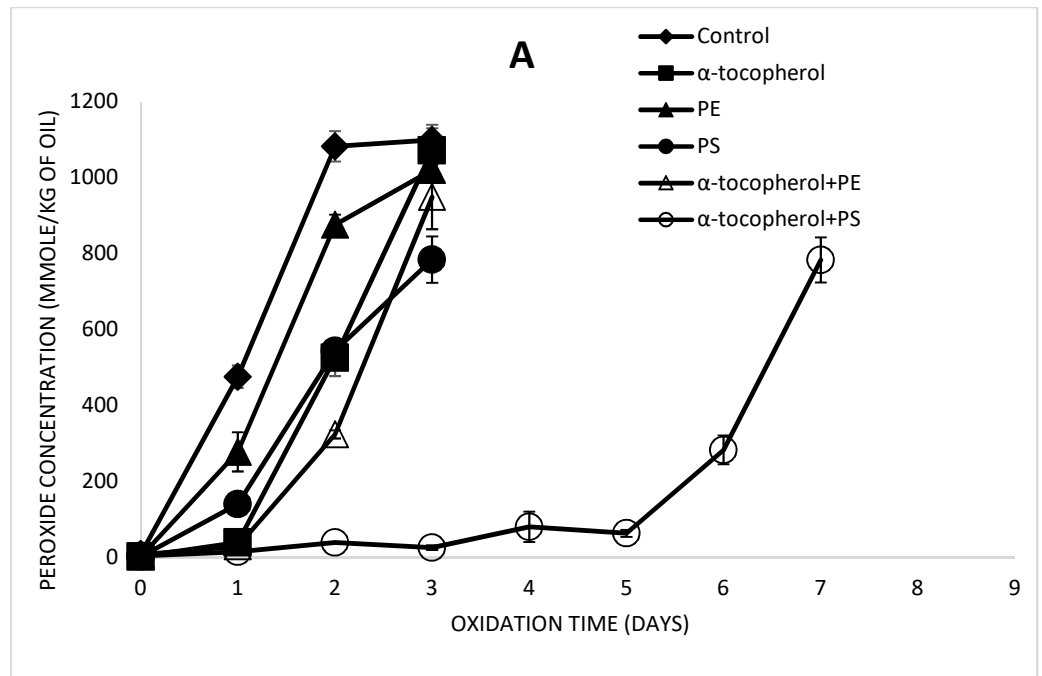
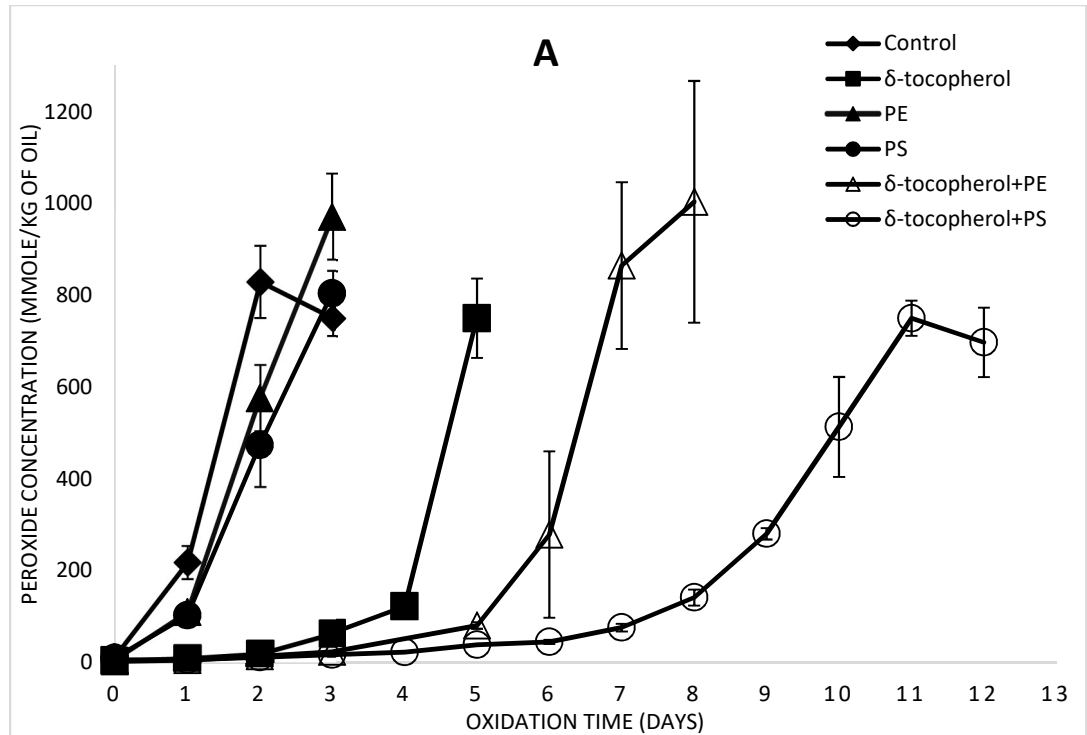


Figure 4.1: Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with Tween 20 containing 3.0 μ mol/kg emulsion α -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS

δ -Tocopherol differs from α -tocopherol in that it has only one methyl group compared to 3 for α -tocopherol meaning that δ -tocopherol is more polar and surface active(Chaiyasit et al., 2005). Addition of δ -tocopherol (3.0 $\mu\text{mol/kg}$ emulsion) to the Tween 20 stabilized emulsions extended the hydroperoxide lag phase to 3 days and hexanal lag phase to 4 days. PE and PS alone again did not change hydroperoxide and hexanal lag phases compared to the control. When PE was added with δ -tocopherol in the emulsion the combination extended the hydroperoxide lag phase to 6 days and hexanal lag phase to 7 days. This produced an interaction index of 1.5 indicating synergism for both hydroperoxides and hexanal. The combination of PS and δ -tocopherol extended the hydroperoxide lag phase to 7 days and hexanal lag phase to 10 days resulting in an interaction index of 1.75 for hydroperoxides and 3 for hexanal.



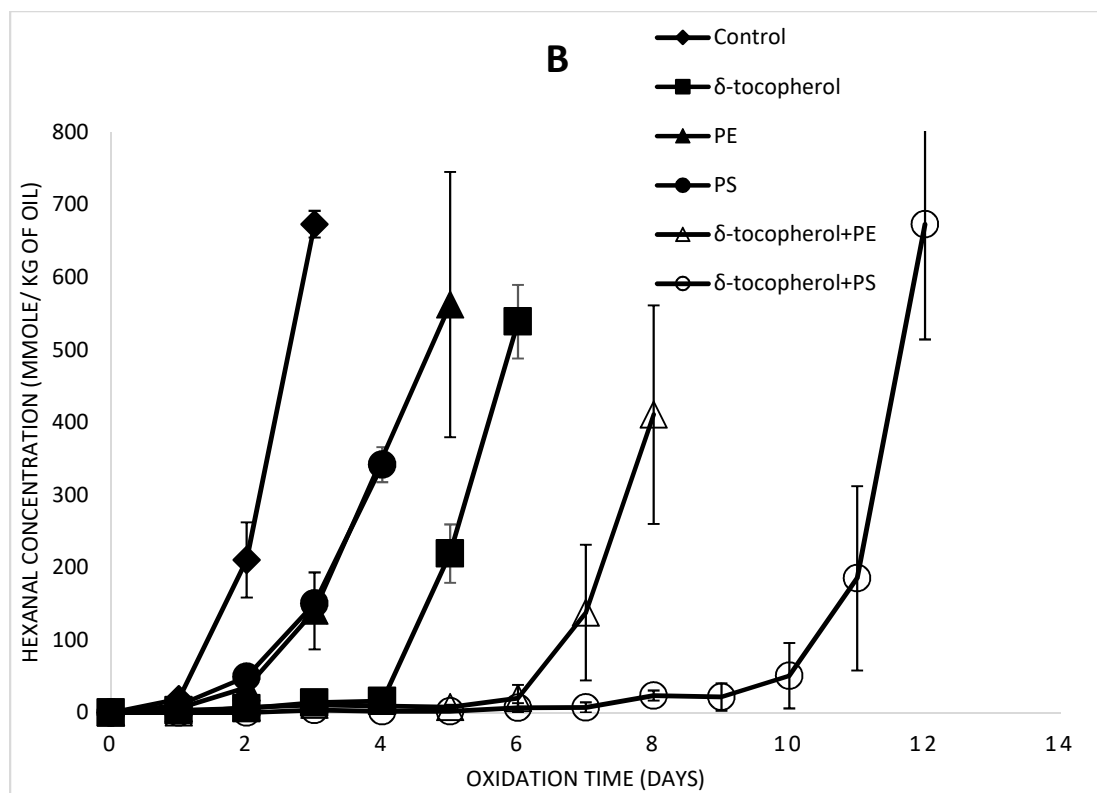


Figure 4.2: Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with Tween 20 containing 3.0 μ mol/kg emulsion δ -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS

α -Tocopherol is available as a food additive but the other tocopherol homologues like δ -tocopherol are currently too expensive for use in foods. In order to take advantage of the unique properties of the other tocopherol homologues, the industry often uses mixed tocopherol, a by-product of oil refining (Shahidi & de Camargo, 2016). Addition of mixed tocopherols (3.0 μ mol/kg emulsion) to the Tween 20 stabilized emulsions by itself extended the hydroperoxide and hexanal lag phases to 3 days. PE and PS alone again did not change hydroperoxide and hexanal lag phases compared to the control. When PE was added with mixed tocopherol in the emulsion it extended both hydroperoxide and hexanal lag phases to 5 days and resulted in an interaction index of 1.67 for hydroperoxides and 1.33 for hexanal. The combination of PS and mixed tocopherol extended

the hydroperoxide lag phase to 5 days and hexanal lag phase to 6 days, an interaction index of 1.25 and 3, respectively.

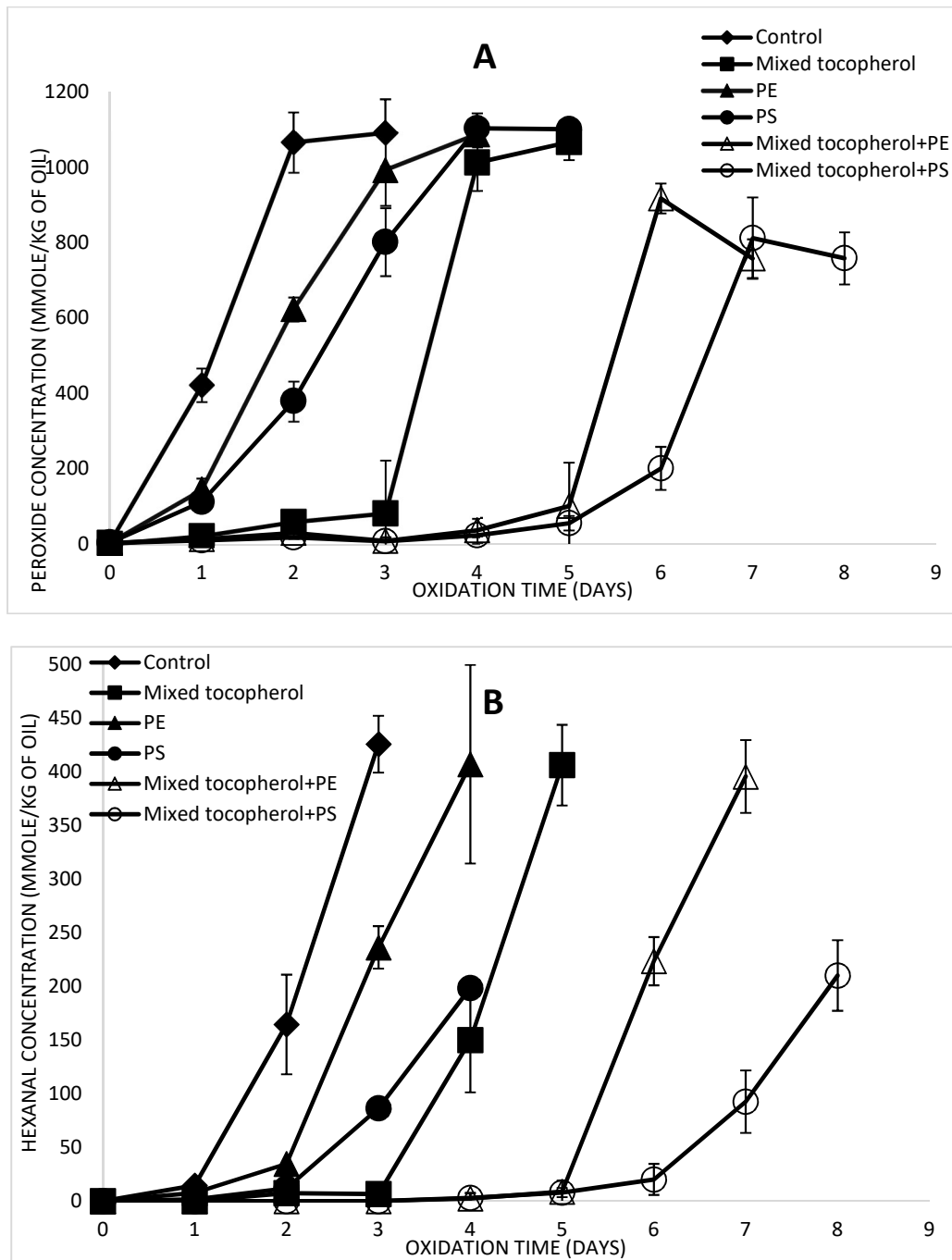


Figure 4.3: Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with Tween 20 containing 3.0 μ mol/kg emulsion mixed tocopherol and/or 15.0 μ mol/kg emulsion PE or PS

4.2.2. Impact of PE and PS with or without tocopherols on the oxidation of BSA stabilized SSO O/W emulsions

In oil-in-water emulsions, tocopherols are primarily found in the emulsion droplet because tocopherols have essentially zero water solubility (Dubbs & Gupta, 1998). Phospholipids could be found in the lipid droplet, at the droplet interface and suspended in structures like micelles in the continuous phase. Protein and small molecule surfactant (e.g. Tweens)-stabilized emulsions could have different impacts on antioxidant activity by impacting the physical location of the tocopherol and phospholipid which in turn could impact their ability to synergistically inhibit lipid oxidation. For example, emulsifiers could impact interactions between tocopherols and phospholipids due to differences in their surface charge (-8.5 mV for Tween 20 vs -33 mV for BSA) which could change the location of the phospholipids through charge repulsion. In addition, proteins could form thicker emulsion droplet interfaces (Berton-Carabin, Ropers, & Genot, 2014) that could impact interactions between tocopherol in the droplet and phospholipids in the continuous phase. Tweens are also known to form micelles in the continuous phase of emulsion that can solubilize tocopherols out of the emulsion droplet which could also impact their ability to interact with phospholipids (Kiralan et al., 2014). Due to these potential differences, synergism between tocopherols and phospholipids were also determined in BSA-stabilized oil-in-water emulsions.

The BSA-stabilized SSO emulsions had a lag phase for lipid hydroperoxides formation of 0 days and a lag phase of hexanal formation of 1 day. Addition of α -tocopherol (3.0 $\mu\text{mol/kg}$ emulsion) to the emulsions extended the hydroperoxide lag phase to 3 days and increased the hexanal lag phase to 4 days. PE alone extended the hydroperoxide lag phase to 1 day and the hexanal lag phase to 4 days whereas PS alone did not affect the hydroperoxide lag phase and increased the hexanal lag phase to 4 days. When PE was added with α -tocopherol to the BSA-stabilized emulsion it extended the hydroperoxide lag phase to 4 days the hexanal lag phase to 7

days which produced interaction index of 1 for both indicating an additive effect. The combination of PS and α -tocopherol extended the hydroperoxide lag phase to 4 days and hexanal lag phase to 8 days which produced interaction indexes of 1.3 and 1.2 respectively.

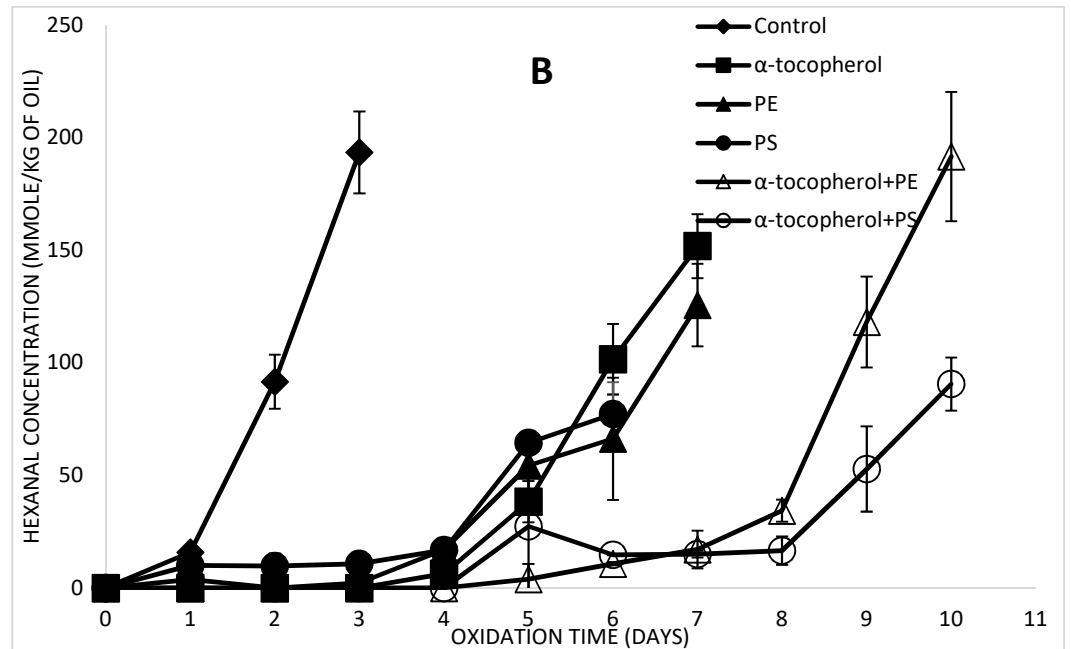
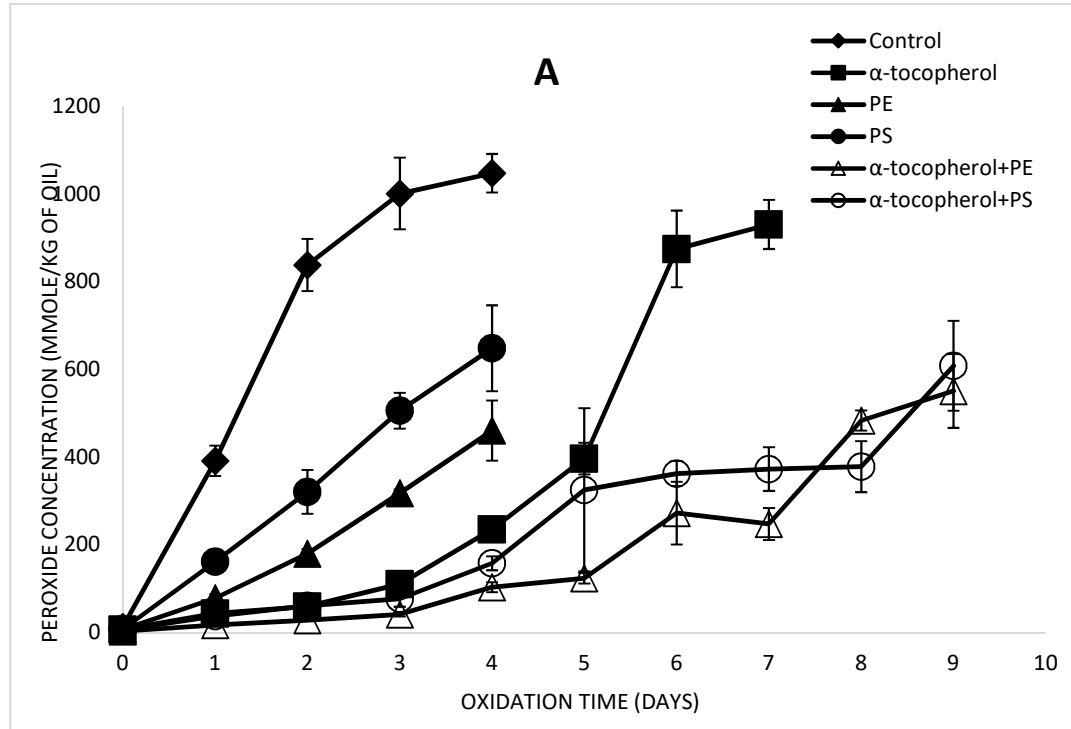
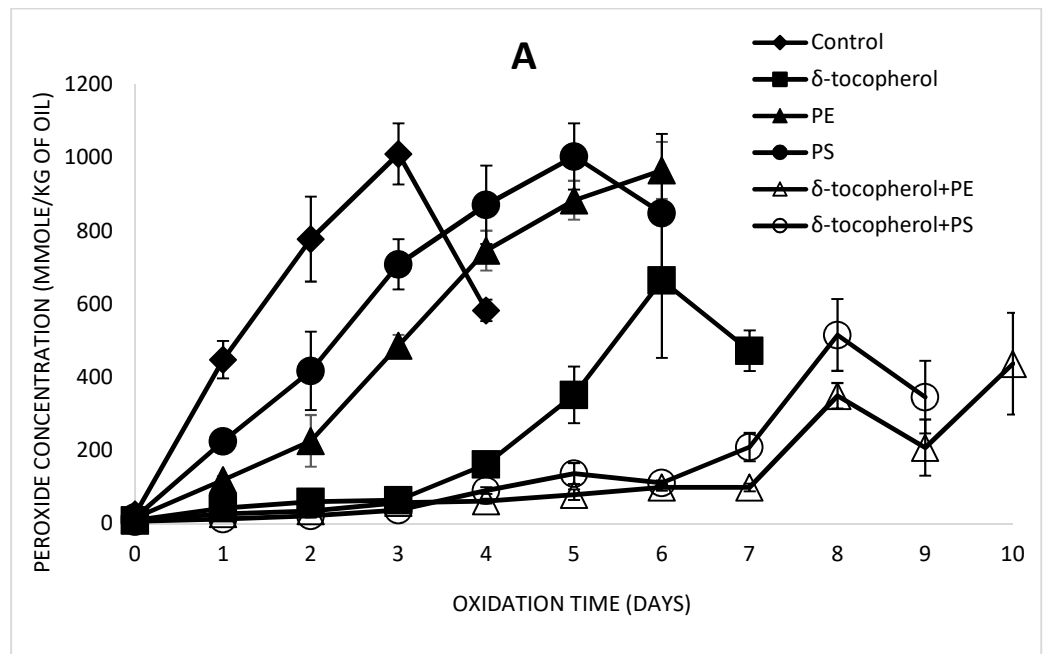


Figure 4.4: Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with bovine serum albumin (BSA) containing 3.0 μ mol/kg emulsion α -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS

Addition of δ -tocopherol (3.0 μ mol /kg emulsion) to the BSA-stabilized oil-in-water emulsion extended the hydroperoxide lag phase to 4 days and hexanal lag phase to 5 days. PE alone extended the hydroperoxide lag phase to 1 day and the hexanal lag phase to 3 days and PS alone extended the hydroperoxide lag phase to 1 day and hexanal lag phases to 2 days. When PE was added with δ -tocopherol in the emulsion it extended the hydroperoxide lag phase to 7 days and hexanal lag phase to 11 days which produced interaction indexes of 1.4 and 1.7 respectively. The combination of PS and δ -tocopherol extended the hydroperoxide lag phase to 6 days and hexanal lag phase to 10 days which produced interaction indexes of 1.2 and 1.8 respectively.



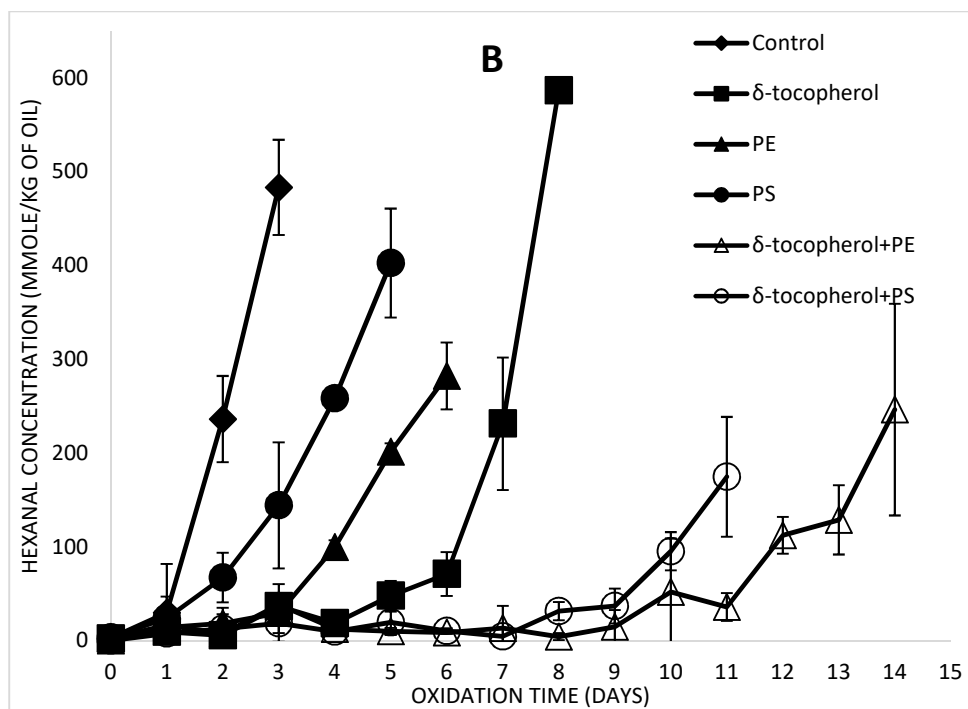


Figure 4.5: Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with bovine serum albumin (BSA) containing 3.0 μ mol/kg emulsion δ -tocopherol and/or 15.0 μ mol/kg emulsion PE or PS

Addition of 3.0 μ mol mixed tocopherol/kg emulsion extended both the lag phases to 3 days. PE alone extended the hydroperoxide lag phase to 1 day and the hexanal lag phase to 3 days whereas PS alone extended the hydroperoxide lag phase to 1 day and hexanal lag phases to 2 days. When PE was added with mixed tocopherol in the emulsion it extended the hydroperoxide lag phase to 5 days and the hexanal lag phase to 7 days which produced interaction indexes of 1.3 and 1.5 respectively. The combination of PS and mixed tocopherol extended the hydroperoxide lag phase to 4 days and the hexanal lag phase to 5 days which produced interaction indexes of 1.3 and 2.5 respectively.

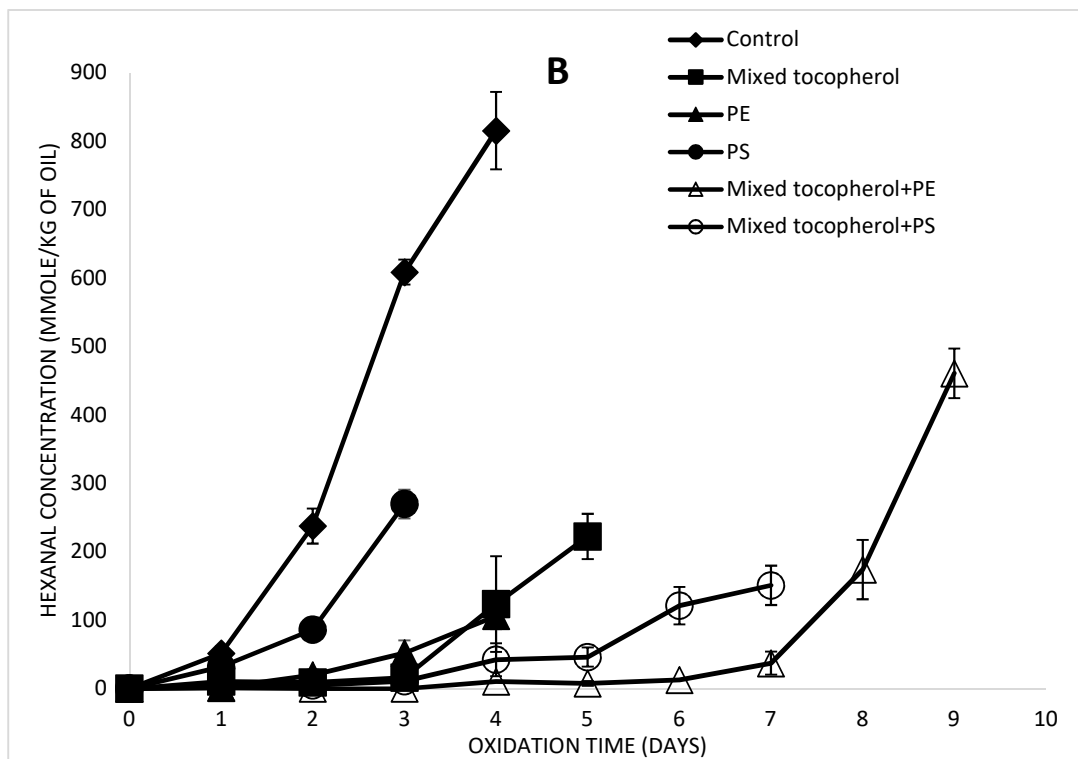
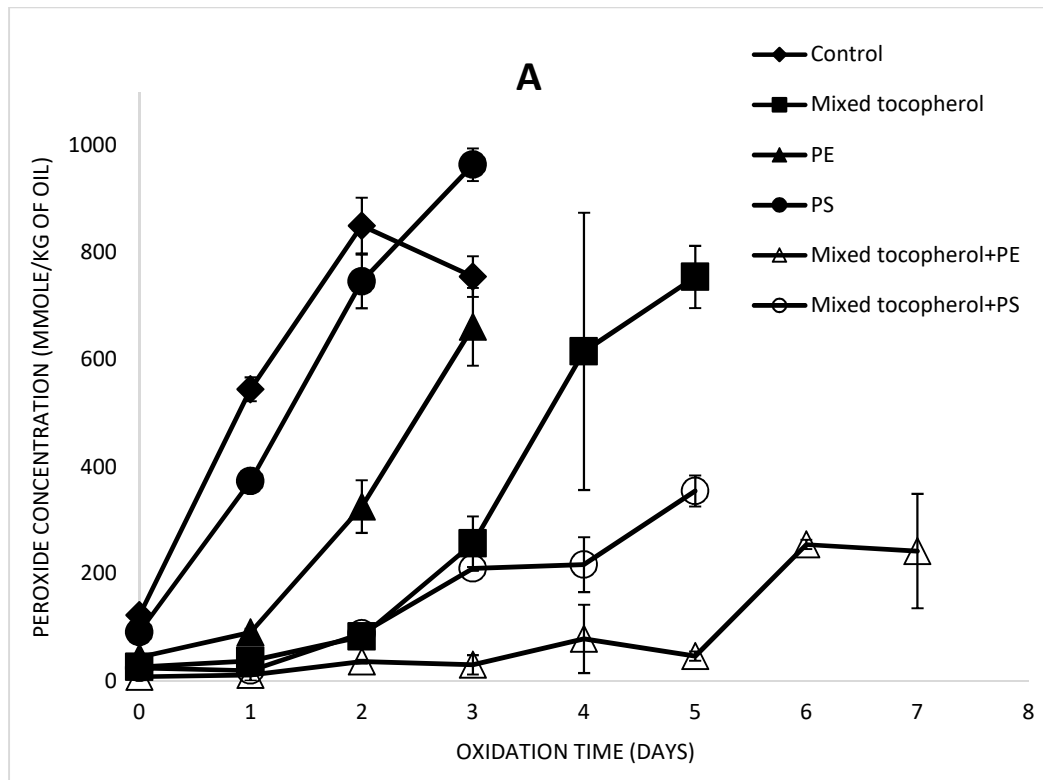


Figure 4.6: Formation of lipid hydroperoxides (A) and hexanal (B) in 1% stripped soybean oil-in-water emulsions stabilized with bovine serum albumin (BSA) containing 3.0 μ mol/kg emulsion mixed tocopherol and/or 15.0 μ mol/kg emulsion PE or PS

4.3. Summary of Interaction index

sample	interaction index			
	Tween 20		BSA	
	hydroperoxide	hexanal	hydroperoxide	hexanal
α -tocopherol+ PE	1 (additive)	1 (additive)	1 (additive)	1 (additive)
α -tocopherol+ PS	5 (synergistic)	5 (synergistic)	1.33 (synergistic)	1.17 (synergistic)
δ -tocopherol+ PE	1.5 (synergistic)	1.5 (synergistic)	1.4 (synergistic)	1.67 (synergistic)
δ -tocopherol+ PS	1.75 (synergistic)	3 (synergistic)	1.2 (synergistic)	1.8 (synergistic)
mixed tocopherol+ PE	1.67 (synergistic)	1.33 (synergistic)	1.25 (synergistic)	1.5 (synergistic)
mixed tocopherol+ PS	1.25 (synergistic)	3 (synergistic)	1.33 (synergistic)	2.5 (synergistic)

Table 4.2: Interaction index between tocopherols and PE or PS in 1% stripped soybean oil-in-water emulsions stabilized with either Tween 20 or bovine serum albumin (BSA).

4.4. Summary of oxidation lag phases

Sample	Lag Phase			
	Tween 20		BSA	
	Hydroperoxide	Hexanal	Hydroperoxide	Hexanal
Control	0	1	0	1
PE	0	1	1	3

PS	1	1	1	2
α -tocopherol	1	2	3	4
α -tocopherol+ PE	1	2	4	7
α -tocopherol+ PS	5	6	4	8
δ -tocopherol	3	4	4	4
δ -tocopherol+ PE	6	7	7	11
δ -tocopherol+ PS	7	10	6	10
Mixed	3	3	3	3
Mixed tocopherol+ PE	5	5	5	7
Mixed tocopherol+ PS	5	6	4	5

Table 4.3: Summary of hydroperoxide and hexanal lag phases in 1% SSO oil-in-water emulsion stabilized either with Tween 20 or BSA

4.5. Discussion

In oil-in-water emulsions, nonpolar antioxidants are more effective as defined in the antioxidant polar paradox hypothesis (Porter et al., 1989). More recently, the best antioxidants in oil-in-water emulsions are thought to be not only nonpolar and retained in the emulsions droplet but also surface active so they partition at the oil-water interface where lipid oxidation is prevalent. Tocopherol homologues are nonpolar and have no water solubility. The tocopherol homologues vary in surface activity with δ -tocopherol being the more surface active than α -tocopherol (Chaiyasit et al., 2005). It has been reported previously that the more surface active tocopherols were more effective in oil-in-water emulsions (Chaiyasit et al., 2005; HUANG et al., 1994; Wagner et al., 2004). Inhibition of lipid oxidation in this study reflects the current hypothesis that the most surface active tocopherols are most effective in oil-in-water emulsions. When the

tocopherols were added to the emulsion by themselves, antioxidant activity was in the order of $\delta \geq \text{mixed tocopherols} > \alpha$. The mixed tocopherols contained only 8.5% α -tocopherol making it more surface active than α -tocopherol alone which could explain its higher antioxidant activity. Mixed tocopherols were less surface active than δ -tocopherol but two had similar antioxidant activities with δ -tocopherol being slightly better. Mixed tocopherols have been postulated to be better antioxidants than individual tocopherols because the mixtures can partition into multiple locations thus placing more antioxidant near sites of free radical generation. This could be why δ -tocopherol was not a dramatically better antioxidant than the mixed tocopherols.

Overall, the best antioxidant activity was observed with combinations of the most surface active tocopherols and phospholipids. For example, δ -tocopherol produced longer lag phases for both hydroperoxide and hexanal formation than α -tocopherol and the mixed tocopherols in the presence of both PE and PS. The phospholipids are surface active and this was observed in the reduction of zeta potential by PS in the Tween 20-stabilized oil-in-water emulsion. The ability of PS but not PE to decrease zeta potential in the Tween emulsions could be due to the great negative charge of PS compared to PE as seen in lipid vesicles (Roy, Gallardo, & Estelrich, 1998) and the greater negative charge of PS compared to Tween 20 in oil-in-water emulsions at pH 7.0 (Cardenia et al., 2011; Roy et al., 1998). PS did not change surface charge in the protein stabilized emulsions. This does not necessarily mean that it was not able to concentrate at the surface of the BSA-stabilized emulsions since the BSA and PS could have similar negative charges at pH 7.0.

The combination of phospholipids and tocopherols resulted in synergistic antioxidant activity (interaction index > 1.0) with the exception of α -tocopherol and PE. Again, the most surface active tocopherol were the most effective when used in combination with PE and PS. This

suggests that the phospholipids and tocopherol combinations were most effective when both concentrated at the emulsion droplet interface. Conversely, the inability of α -tocopherol to produce synergistic activity could be due to its lower surface activity and thus less interactions with phospholipids.

Synergistic activity between PS and the tocopherols was greater in the Tween-stabilized emulsions than PE and tocopherols whereas both had a similar activity in the BSA-stabilized emulsions. This again could be due to differences in interfacial concentrations. PS is more negatively charged than PE (Roy et al., 1998). Since BSA is also negatively charged at pH 7.0, it's possible that charge repulsion could decrease the concentration of PS at the interface decreasing its ability to interact with tocopherols.

The synergistic activity between the phospholipids and tocopherols could be due to several factors. Phospholipids have been reported to inhibit lipid oxidation by metal chelation (Cardenia et al., 2011; Dacaranhe & Terao, 2001; Yoon, 1987). Chelators can decrease the ability of transition metals to decompose lipid hydroperoxide into free radicals. Decreased production of free radicals will decrease tocopherol degradation meaning that it can be an effective antioxidant for longer periods of time. PE and PS can also convert the oxidized form of tocopherols, the quinone, back to tocopherol regenerating tocopherols back to their active state. Regeneration of α -tocopherol by PE resulted in synergistic antioxidant in bulk oil (Cui et al., 2015; Doert et al., 2012). The observation that the synergistic activity of tocopherol and PE and PS combinations was greatest when they were both at the interface suggests that they need interact with each other to allow tocopherol regeneration. While it is difficult to know the exact reasons for the observed synergistic activity, the results of this work suggest that regeneration is involved.

4.6. Conclusion

This is the first research that shows that the combination of tocopherols and PE or PS produce synergistic antioxidant activity in oil-in-water emulsions. Antioxidant combinations were able to increase the lag phase of lipid oxidation from 1.3 to 2.75fold. This suggests that the combination of tocopherols and PE or PS could be a good clean label antioxidant strategy. Mixed tocopherols would be the most viable commercial strategy since it was more effective than α -tocopherol and δ -tocopherol is currently too expensive for food applications. The biggest challenge in making this strategy viable in foods will be identifying economical source of PE and PS. High PE lecithins are commercially available but they have not been tested for their ability to synergistically interact with tocopherols.

CHAPTER 5

CONCLUSION AND FUTURE WORK

We found that more polar tocopherols had a stronger antioxidant effect in oil-in-water emulsions. PS acted synergistically and increased the antioxidant activity of α , δ and mixed tocopherol in oil-in-water emulsions stabilized either by Tween 20 or BSA. Whereas, PE could act synergistically with δ and mixed tocopherol only and had an additive antioxidant effect with α -tocopherol. This possibly indicates that antioxidants located at the interface (PS is more surface active than PE) might have a stronger antioxidant effect.

Pure phospholipids can increase the antioxidant activity of tocopherols by 1.5 to 3 times and the combination can be used as a clean label antioxidant strategy, but there is no economical source of pure PE or PS available yet. Modified lecithin with high PE levels are available, but they have not been tested for their ability to act synergistically with tocopherols in oil-in-water emulsions.

BIBLIOGRAPHY

- Akoh, C. C. (2008). *Food lipids* (3. ed. ed.). New York <>: CRC Press.
- Alaiz, M., Zamora, R., & Hidalgo, F. J. (1996). Contribution of the formation of oxidized lipid amino acid reaction products to the protective role of amino acids in oils and fats. *Journal of Agricultural and Food Chemistry*, 44(7), 1890-1895. doi:10.1021/jf9502919
- Bandarra, N., Campos, R., Batista, I., Nunes, M., & Empis, J. (1999). Antioxidant synergy of α -tocopherol and phospholipids. *Journal of the American Oil Chemists' Society*, 76(8), 905-913. doi:10.1007/s11746-999-0105-4
- Berton-Carabin, C. C., Ropers, M., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. *Comprehensive Reviews in Food Science and Food Safety*, 13(5), 945-977. doi:10.1111/1541-4337.12097
- Brett, R., & Rumsby, M. G. (1994). Susceptibility of myelin glycerophospholipids and sphingolipids to oxidative attack by hydroxyl free-radicals as measured by the thiobarbituric acid test. *Neurochemistry International*, 24(3), 241-251. doi:10.1016/0197-0186(94)90081-7
- Cardenia, V., Waraho, T., Rodriguez-Estrada, M., Julian McClements, D., & Decker, E. (2011). Antioxidant and prooxidant activity behavior of phospholipids in stripped soybean oil-in-water emulsions. *Journal of the American Oil Chemists' Society*, 88(9), 1409-1416. doi:10.1007/s11746-011-1807-y
- Carlotti, M. E., Gallarate, M., Gasco, M. R., Morel, S., Serafino, A., & Ugazio, E. (1997). *Synergistic action of vitamin C and amino acids on vitamin E in inhibition of the lipoperoxidation of linoleic acid in disperse systems* doi://doi.org/10.1016/S0378-5173(97)00168-3
- Chaiyasit, W., Silvestre, M., McClements, D. J., & Decker, E. A. (2000). Ability of surfactant hydrophobic tail group size to alter lipid oxidation in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 48(8), 3077-3080. doi:10.1021/jf000323e
- Chaiyasit, W., McClements, D. J., & Decker, E. A. (2005). The relationship between the physicochemical properties of antioxidants and their ability to inhibit lipid oxidation in bulk oil and oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 53(12), 4982-4988. doi:10.1021/jf0501239
- Chen, B., McClements, D. J., & Decker, E. A. (2011). Minor components in food oils: A critical review of their roles on lipid oxidation chemistry in bulk oils and emulsions. *Critical Reviews in Food Science and Nutrition*, 51(10), 901-916. doi:10.1080/10408398.2011.606379

- Choe, E., & Min, D. B. (2005). Chemistry and reactions of reactive oxygen species in foods. *Journal of Food Science*, 70(9), R159.
- Choe, E., & Min, D. B. (2009). Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Reviews in Food Science and Food Safety*, 8(4), 345-358. doi:10.1111/j.1541-4337.2009.00085.x
- Cui, L., & Decker, E. A. (2016). Phospholipids in foods: Prooxidants or antioxidants? *Journal of the Science of Food and Agriculture*, 96(1), 18-31. doi:10.1002/jsfa.7320
- Cui, L., McClements, D. J., & Decker, E. A. (2015). Impact of phosphatidylethanolamine on the antioxidant activity of α -tocopherol and trolox in bulk oil. *Journal of Agricultural and Food Chemistry*, 63(12), 3288. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/25768290>
- Dacaranhe, C. D., & Terao, J. (2001). Effect of phosphatidic acid and phosphatidylserine on lipid oxidation in beef homogenate during storage and in emulsified sardine oil. *Journal of Food Science*, 66(3), 422-427. doi:10.1111/j.1365-2621.2001.tb16121.x
- Decker, E. A., McClements, D. J., Bourlieu-Lacanal, C., Durand, E., Figueroa-Espinoza, M. C., Lecomte, J., & Villeneuve, P. (2017). Hurdles in predicting antioxidant efficacy in oil-in-water emulsions. *Trends in Food Science & Technology*, 67, 183-194. doi:10.1016/j.tifs.2017.07.001
- DeSalvo, K. B., Olson, R., & Casavale, K. O. (2016). Dietary guidelines for americans. *Jama-Journal of the American Medical Association*, 315(5), 457-458. doi:10.1001/jama.2015.18396
- Djordjevic, D., Cercaci, L., Alamed, J., McClements, D. J., & Decker, E. A. (2007). Chemical and physical stability of citral and limonene in sodium dodecyl sulfate-chitosan and gum arabic-stabilized oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 55(9), 3585-3591. doi:10.1021/jf063472r
- Doert, M., Jaworska, K., Moersel, J., & Kroh, L. (2012). Synergistic effect of lecithins for tocopherols: Lecithin-based regeneration of α -tocopherol. *European Food Research and Technology*, 235(5), 915-928. doi:10.1007/s00217-012-1815-7
- Dubbs, M. D., & Gupta, R. B. (1998). Solubility of vitamin E (alpha-tocopherol) and vitamin K-3 (menadione) in ethanol-water mixture. *Journal of Chemical and Engineering Data*, 43(4), 590-591. doi:10.1021/je980017l
- FRANKEL, E. N. (1984). Lipid oxidation - mechanisms, products and biological significance. *Journal of the American Oil Chemists Society*, 61(12), 1908-1917. doi:10.1007/BF02540830
- FRANKEL, E. N., HUANG, S. W., KANNER, J., & GERMAN, J. B. (1994). Interfacial phenomena in the evaluation of antioxidants - bulk oils vs emulsions. *Journal of Agricultural and Food Chemistry*, 42(5), 1054-1059. doi:10.1021/jf00041a001

- Gal, S., Pinchuk, I., & Lichtenberg, D. (2003). Peroxidation of liposomal palmitoyllinoleoylphosphatidylcholine (PLPC), effects of surface charge on the oxidizability and on the potency of antioxidants. *Chemistry and Physics of Lipids*, 126(1), 95-110. doi:10.1016/S0009-3084(03)00096-3
- German, J. B. (1999). Food processing and lipid oxidation. *Advances in Experimental Medicine and Biology*, 459, 23-50.
- Graf, E., & Eaton, J. W. (1990). *Antioxidant functions of phytic acid* doi://doi.org/10.1016/0891-5849(90)90146-A
- Haahr, A., & Jacobsen, C. (2008). Emulsifier type, metal chelation and pH affect oxidative stability of n-3-enriched emulsions. *European Journal of Lipid Science and Technology*, 110(10), 949-961. doi:10.1002/ejlt.200800035
- Hu, M., McClements, D. J., & Decker, E. A. (2003). Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. *Journal of Agricultural and Food Chemistry*, 51(6), 1696-1700. doi:10.1021/jf020952j
- HUANG, S. W., FRANKEL, E. N., & GERMAN, J. B. (1994). Antioxidant activity of alpha-tocopherols and gamma-tocopherols in bulk oils and in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 42(10), 2108-2114. doi:10.1021/jf00046a007
- HUANG, S. W., FRANKEL, E. N., & GERMAN, J. B. (1995). Effects of individual tocopherols and tocopherol mixtures on the oxidative stability of corn-oil triglycerides. *Journal of Agricultural and Food Chemistry*, 43(9), 2345-2350. doi:10.1021/jf00057a006
- Jacobsen, C., Hartvigsen, K., Thomsen, M. K., Hansen, L. F., Lund, P., Skibsted, L. H., . . . Meyer, A. S. (2001). Lipid oxidation in fish oil enriched mayonnaise: Calcium disodium ethylenediaminetetraacetate, but not gallic acid, strongly inhibited oxidative deterioration. *Journal of Agricultural and Food Chemistry*, 49(2), 1009-1019. doi:10.1021/jf000729r
- Johnson, D. R., & Decker, E. A. (2015). The role of oxygen in lipid oxidation reactions: A review. *Annual Review of Food Science and Technology*, Vol 6, 6, 171-190. doi:10.1146/annurev-food-022814-015532
- Johnson, D. R., Tian, F., Roman, M. J., Decker, E. A., & Goddard, J. M. (2015). Development of iron-chelating poly(ethylene terephthalate) packaging for inhibiting lipid oxidation in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 63(20), 5055. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/25985711>
- KamalEldin, A., & Appelqvist, L. A. (1996a). The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*, 31(7), 671-701. doi:10.1007/BF02522884
- KamalEldin, A., & Appelqvist, L. A. (1996b). The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*, 31(7), 671-701. doi:10.1007/BF02522884

- Kanematsu, H., Morise, E., Niiya, I., Imamura, M., Matsumoto, A., & Katsui, G. (1972). Influence of tocopherols on oxidative stability of margarines. *J Jpn Soc Food Nutr*, 25, 343-348.
- KASHIMA, M., CHA, G. S., ISODA, Y., HIRANO, J., & MIYAZAWA, T. (1991). The antioxidant effects of phospholipids on perilla oil. *Journal of the American Oil Chemists Society*, 68(2), 119-122. doi:10.1007/BF02662331
- KING, C. C., JR. (1986). *Effects of antioxidants and modified atmospheres on the storage stability of the pecan kernel (tocopherol, vacuum, nitrogen flush, food quality)* Available from Dissertations & Theses Europe Full Text: Science & Technology.
- Kiralan, S. S., Doğu-Baykut, E., Kittipongpittaya, K., McClements, D. J., & Decker, E. A. (2014). Increased antioxidant efficacy of tocopherols by surfactant solubilization in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 62(43), 10561. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/25299347>
- Kittipongpittaya, K., Panya, A., Phonsatta, N., & Decker, E. A. (2016). Effects of environmental pH on antioxidant interactions between rosmarinic acid and α -tocopherol in oil-in-water (O/W) emulsions. *Journal of Agricultural and Food Chemistry*, 64(34), 6575. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/27494424>
- Laguerre, M., Giraldo, L. J. L., Lecomte, J., Figueroa-Espinoza, M., Barea, B., Weiss, J., . . . Villeneuve, P. (2009). Chain length affects antioxidant properties of chlorogenate esters in emulsion: The cutoff theory behind the polar paradox. *Journal of Agricultural and Food Chemistry*, 57(23), 11335-11342. doi:10.1021/jf9026266
- Laguerre, M., Giraldo, L. J. L., Lecomte, J., Figueroa-Espinoza, M., Barea, B., Weiss, J., . . . Villeneuve, P. (2010). Relationship between hydrophobicity and antioxidant ability of "phenolipids" in emulsion: A parabolic effect of the chain length of rosmarinate esters. *Journal of Agricultural and Food Chemistry*, 58(5), 2869-2876. doi:10.1021/jf904119v
- Martín, J. M. P., Freire, P. F., Daimiel, L., Martínez-Botas, J., Sánchez, C. M., Lasunción, M. Á, . . . Hazen, M. J. (2014). *The antioxidant butylated hydroxyanisole potentiates the toxic effects of propylparaben in cultured mammalian cells* doi://doi-org.silk.library.umass.edu/10.1016/j.fct.2014.07.031
- McClements, D. J. (2008). Lipid-based emulsions and emulsifiers. dalam. *Food Lipids: Chemistry, Nutrition, and Biotechnology*, , 97.
- Melo, P. S., Arrivetti, Leandro de Oliveira Rodrigues, Alencar, S. M. d., & Skibsted, L. H. (2016). *Antioxidative and prooxidative effects in food lipids and synergism with α -tocopherol of açai seed extracts and grape rachis extracts* doi://doi.org/10.1016/j.foodchem.2016.06.101
- Min, D. B., & Boff, J. M. (2002). Chemistry and reaction of singlet oxygen in foods. *Comprehensive Reviews in Food Science and Food Safety*, 1(2), 58-72. doi:10.1111/j.1541-4337.2002.tb00007.x

- Naumov, V. V., & Vasilev, R. F. (2003). Antioxidant and prooxidant effects of tocopherol. *Kinetics and Catalysis*, 44(1), 101-105. doi:10.22528919697
- Nieva-Echevarría, B., Manzanos, M. J., Goicoechea, E., & Guillén, M. D. (2015). 2,6-di-tert-butylhydroxytoluene and its metabolites in foods. *Comprehensive Reviews in Food Science and Food Safety*, 14(1), 67-80. doi:10.1111/1541-4337.12121
- Peyrat-Maillard, M., Cuvelier, M., & Berset, C. (2003). Antioxidant activity of phenolic compounds in 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: Synergistic and antagonistic effects. *Journal of the American Oil Chemists' Society*, 80(10), 1007-1012. doi:10.1007/s11746-003-0812-z
- Pokorny, J. (2007). Are natural antioxidants better - and safer - than synthetic antioxidants? (vol 109, pg 629, 2007). *European Journal of Lipid Science and Technology*, 109(8), 883. doi:10.1002/ejlt.200790023
- Porter, W. L., Black, E. D., & Drolet, A. M. (1989). Use of polyamide oxidative fluorescence test on lipid emulsions: Contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *Journal of Agricultural and Food Chemistry*, 37(3), 615-624. doi:10.1021/jf00087a009
- Roy, M. T., Gallardo, M., & Estelrich, J. (1998). Influence of size on electrokinetic behavior of phosphatidylserine and phosphatidylethanolamine lipid vesicles. *Journal of Colloid and Interface Science*, 206(2), 512-517. doi:10.1006/jcis.1998.5715
- Saini, R. K., & Keum, Y. (2016). *Tocopherols and tocotrienols in plants and their products: A review on methods of extraction, chromatographic separation, and detection* doi://doi.org/10.1016/j.foodres.2016.01.025
- Shahidi, F., & de Camargo, A. (2016). Tocopherols and tocotrienols in common and emerging dietary sources: Occurrence, applications, and health benefits. *International Journal of Molecular Sciences*, 17(10), 1745. doi:10.3390/ijms17101745
- SHANTHA, N. C., & DECKER, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International*, 77(2), 421-424.
- Shimajiri, J., Shiota, M., Hosokawa, M., & Miyashita, K. (2013). Synergistic antioxidant activity of milk sphingomyeline and its sphingoid base with alpha-tocopherol on fish oil triacylglycerol. *Journal of Agricultural and Food Chemistry*, 61(33), 7969-7975. doi:10.1021/jf401788j
- Silvestre, M., Chaiyasit, W., Brannan, R. G., McClements, D. J., & Decker, E. A. (2000). Ability of surfactant headgroup size to alter lipid and antioxidant oxidation in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 48(6), 2057-2061. doi:10.1021/jf991162l

- Viani, P., Cervato, G., Fiorilli, A., Rigamonti, E., & Cestaro, B. (1990). Studies on peroxidation processes of model membranes and synaptosomes - role of phosphatidic-acid. *Chemistry and Physics of Lipids*, 52(1), 49-55. doi:10.1016/0009-3084(90)90006-D
- Wagner, K., Isnardy, B., & Elmadfa, I. (2004). Γ - and δ -tocopherols are more effective than α -tocopherol on the autoxidation of a 10% rapeseed oil triacylglycerol-in-water emulsion with and without a radical initiator. *European Journal of Lipid Science and Technology*, 106(1), 44-51. doi:10.1002/ejlt.200300885
- Waraho, T., McClements, D. J., & Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science & Technology*, 22(1), 3-13. doi:10.1016/j.tifs.2010.11.003
- YAMAUCHI, R., YAGI, Y., & KATO, K. (1996). Oxidation of .ALPHA.-tocopherol during the peroxidation of dilinoleoylphosphatidylcholine in liposomes. *Bioscience, Biotechnology, and Biochemistry*, 60(4), 616-620. doi:10.1271/bbb.60.616
- Yoon, S. H. (1987). Roles of phospholipids in flavor stability of soybean oil. *Kor J Food Sci Technol*, 19, 28.