



**DEVELOPMENT OF POLY(LACTIC ACID) FILMS
INCORPORATED WITH ALMOND SKIN EXTRACT**

SAHRANUR TABAKOĞLU

Master's Thesis

Graduate School
Izmir University of Economics

Izmir

2021

**DEVELOPMENT OF POLY(LACTIC ACID) FILMS
INCORPORATED WITH ALMOND SKIN EXTRACT**

SAHRANUR TABAKOĐLU

A Thesis Submitted to
The Graduate School of Izmir University of Economics
Master's Program in Bioengineering

Izmir

2021

ABSTRACT

DEVELOPMENT OF POLY(LACTIC ACID) FILMS INCORPORATED WITH ALMOND SKIN EXTRACT

Tabakođlu, Sahranur

Master's Program in Bioengineering

Advisor: Asst. Prof. Dr. Işınay Ebru Yüzay

July, 2021

In this thesis, it was aimed to develop a functional composite polymer film using natural extract. For this purpose, almond skin was used as a natural antioxidant source. Antioxidant polyphenolic compounds were extracted from the almond skin. For the polymer matrix, poly(lactic acid) (PLA) which is biobased and biodegradable was used. Prepared almond skin extract (ASE) was incorporated into PLA solution and three different extract concentrations as 1.8, 3.2, and 4.6 wt.% were used to prepare PLA-ASE film samples. To develop the films, the solvent casting method was used. After the formation of the PLA-ASE film samples, the structural, gas barrier, thermal,

mechanical, optical, surface morphology properties, and antioxidant activities were analyzed. While the extract addition improves the oxygen barrier of the films independently of the extract content, caused a slight decrease in the water vapor barrier as related to the ASE content. Additionally, the ultraviolet-visible light barrier of the neat PLA (without ASE) film significantly improved with the addition of the almond skin extract. Differential scanning calorimetry (DSC) results showed the almond skin extract did not affect the thermal transition points of the films. In mechanical properties, a decrease was observed depending on the almond skin extract content. To investigate the antioxidant activity of the films, DPPH radical scavenging activity (RSA) analysis was done and significant RSA was observed. Based on the results of the study, promising functional composite polymer films were developed for functional food packaging applications.

Keywords: poly(lactic acid), almond skin extract, biobased film characterization, functional packaging

ÖZET

BADEM ZARI EKSTRAKTI İLAVESİ İLE POLİ(LAKTİK ASİT) FİMLERİN GELİŞTİRİLMESİ

Tabakođlu, Sahranur

Biyomühendislik Yüksek Lisans Programı

Tez Danışmanı: Dr. Öğr. Üyesi Işınay Ebru Yüzay

Temmuz, 2021

Bu tezde, doğal kaynaklı bir ekstrakt kullanılarak fonksiyonel bir kompozit polimer film geliştirilmesi amaçlanmıştır. Bu amaçla doğal bir antioksidan kaynağı olarak badem zarı kullanılmıştır. Badem zarından antioksidan özellik gösteren polifenolik bileşiklerin ekstraksiyonu yapılmıştır. Polimer matrisi için ise doğal hammaddeden üretilen ve biyolojik olarak parçalanabilen poli(laktik asit) kullanılmıştır. Hazırlanan badem zarı ekstraktı (BZE) PLA çözeltisine eklendi ve PLA-BZE film örnekleri hazırlamak için ağırlıkça %1.8, 3.2, ve 4.6 olmak üzere üç farklı ekstrakt konsantrasyonu kullanıldı. Filmleri oluşturmak için solvent döküm yöntemi kullanıldı.

PLA-BZE film örneklerinin oluşturulmasından sonra yapısal, gaz bariyeri, termal, mekanik, optik, yüzey morfolojisi özellikleri ve antioksidan aktiviteleri analiz edildi. BZE ilavesi, ekstrakt miktarından bağımsız olarak filmlerin oksijen geçirgenliğini düşürürken, su buharı geçirgenliği özelliğinde ekstrakt miktarıyla ilişkili olarak bir artışa neden olmuştur. Ek olarak, saf PLA (BZE ilavesiz) filminin ultraviyole-görünür ışık bariyeri, badem zarı ekstresinin eklenmesiyle önemli ölçüde iyileştirildi. Diferansiyel taramalı kalorimetri (DSC) sonuçları, badem zarı ekstresinin kompozit filmlerin termal geçiş noktalarını etkilemediğini gösterdi. Mekanik özelliklerde ise badem zarı ekstresi miktarına bağlı olarak bir azalma gözlenmiştir. Filmlerin antioksidan aktivitesini araştırmak için DPPH radikal yakalayıcı aktivite (RSA) analizi yapıldı ve anlamlı ölçüde RSA gözlemlendi. Tüm sonuçlar göz önünde bulundurulduğunda, fonksiyel gıda ambalaj uygulamaları için umut veren fonksiyonel kompozit polimer filmler geliştirilmiştir.

Anahtar kelimeler: poli(laktik asit), badem zarı ekstraktı, biyobazlı film karakterizasyonu, fonksiyonel ambalaj

ACKNOWLEDGEMENTS

Firstly, I would like to thank my advisor Asst. Prof. Dr. Işınay Ebru Yüzay for her contributions to the development of this thesis by her knowledge and experiences.

Secondly, I would like to express my sincere thanks to my best friend and companion Zeynep Altan who is one of my biggest supporters. She has been there for me whenever I needed her. I am very lucky to have the precious friendship offered by her beautiful heart.

I am so thankful to my dear beloved Çağatay Kırmızıay, who has been my motivation source almost during all of my master's education. He also helped me to the best of his ability with any problem I needed to solve. Furthermore, believing more than me that I can succeed, he has always supported me and calmed me even in my most stressful times. His presence in my life is very precious to me.

Finally, I would like to express my greatest thanks to my dear and beautiful family, my mother Ayten Tabakoğlu, my father Orbay Tabakoğlu, and my sister Simay Tabakoğlu, for their endless support. As always, they stood by me in this period of my life with their unconditional love and I am so grateful for that.

TABLE OF CONTENTS

ABSTRACT.....	iii
ÖZET.....	v
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: LITERATURE REVIEW	5
2.1 <i>Poly(lactic acid)</i>	5
2.1.1 <i>From lactic acid to poly(lactic acid)</i>	6
2.1.2 <i>Some of the properties of PLA</i>	8
2.1.2.1 <i>Mechanical properties</i>	8
2.1.2.2 <i>Thermal properties</i>	9
2.1.2.3 <i>Barrier properties</i>	11
2.1.2.5 <i>Optical properties</i>	12
2.1.3 <i>Major applications of PLA</i>	13
2.1.3.1 <i>Medical industry</i>	15
2.1.3.2 <i>Food industry</i>	16
2.1.3.3 <i>Other application areas</i>	17
2.1.3.4 <i>Advantages and disadvantages of using PLA</i>	18
2.2 <i>Poly(lactic acid) composites</i>	20
2.2.1 <i>Reinforced PLA with fibers and fillers</i>	20
2.2.2 <i>Incorporation of plant-based polyphenolic compounds into PLA</i>	23
2.3 <i>Almond (Prunus dulcis)</i>	26
2.3.1 <i>Almond as a valuable agricultural product</i>	26
2.3.2 <i>Structural composition of almond</i>	28
CHAPTER 3: MATERIALS AND METHODS	31
3.1 <i>Materials</i>	31

3.2 Extraction and characterization of polyphenolic compounds from almond skin	31
3.2.1 Extraction of polyphenolic compounds from almond skins	31
3.2.2 Determination of total polyphenolic content (TPC) of almond skin extract	32
3.2.3 Fourier transform infrared (FT-IR) spectroscopy.....	32
3.3 Film preparation	32
3.4 Film characterization methods.....	33
3.4.1 Optical properties	33
3.4.1.1 Ultraviolet-Visible (UV-Vis) light barrier properties.....	33
3.4.1.3 Color	33
3.4.2 Structural analyses (Fourier transform infrared spectroscopy (FT-IR))..	34
3.4.3 Mechanical properties	34
3.4.4 Gas barrier properties	34
3.4.4.1 Water vapor permeability (WVP).....	34
3.4.4.2 Oxygen permeability	35
3.4.5 Radical scavenging activity (RSA).....	36
3.4.6 Thermal properties	37
3.4.7 Morphological analyses.....	37
3.4.7.1 Scanning electron microscopy (SEM).....	37
3.4.7.2 Atomic force microscopy (AFM).....	38
3.4.8 Statistical analysis	38
CHAPTER 4: RESULTS AND DISCUSSION.....	39
4.1 Extraction and determination of polyphenolics from almond skin	39
4.1.1 Polyphenolic compound extraction from almond skin	39
4.1.2 Total polyphenol content (TPC) of almond skin extract.....	39
4.2. Film preparation	40
4.3 Characterization of film samples	41
4.3.1 Optical properties	41
4.3.1.1 Uv-Vis light barrier properties	41

4.3.1.2 <i>Color</i>	43
4.3.2 <i>Structural analyses (FT-IR)</i>	44
4.3.3 <i>Mechanical properties</i>	47
4.3.4 <i>Gas barrier properties</i>	48
4.3.4.1 <i>Water vapor permeability (WVP)</i>	48
4.3.4.2 <i>Oxygen permeability</i>	49
4.3.5 <i>Radical scavenging activity (RSA)</i>	50
4.3.6 <i>Thermal properties</i>	52
4.3.7 <i>Morphological analyses</i>	54
4.3.7.1 <i>SEM</i>	54
4.3.7.2 <i>AFM</i>	57
CHAPTER 5: CONCLUSION.....	60
APPENDICES	84
<i>Appendix A - Calculation of total polyphenolic content in gallic acid equivalent (GAE)</i>	84
<i>Appendix B - Calculation of transparency</i>	85
<i>Appendix C - Calculation of water vapor permeability</i>	86
<i>Appendix D - Calculations for oxygen permeability</i>	89
<i>Appendix E - Calculation of radical scavenger activity</i>	90
<i>Appendix F - Calculation of crystallinity degree</i>	92

LIST OF TABLES

Table 1. Mechanical properties of unoriented and oriented PLA (Source: Lalla and Chugh, 1990; Farah, Anderson and Langer, 2016).....	9
Table 2. Thermal transition points of PLA.	10
Table 3. For the PLA, some commercialized products and industrial application fields (Source: Jamshidian et al., 2010).	14
Table 4. Mostly used fillers for PLA composite material and their effects (Source: Murariu and Dubois, 2016).	22
Table 5. Effects of some different polyphenolic extracts that were studied in the literature on PLA film.	24
Table 6. Polyphenolic compounds in the almond skin (Source: Prgomet et al., 2017).	29
Table 7. Color test results of the PLA and PLA-ASE film samples.....	44
Table 8. Tensile properties (mean \pm SD; n=3) of the neat PLA and PLA-ASE films. Different superscripts in the same row are significantly different (p<0.05).	47
Table 9. Thermal transition points (mean \pm SD; n=2) obtained from DSC of PLA-ASE films. Different superscripts in the same row are significantly different (p<0.05)....	54

LIST OF FIGURES

Figure 1. The representative basic life cycle of PLA (Source: Auras, Harte and Selke, 2004a).....	6
Figure 2. Synthesis routes for PLA (Source: Pang et al., 2010)	8
Figure 3. Comparison of gas permeation coefficients at 30 °C (Source: Hamad et al., 2015).	12
Figure 4. Main subclasses of polyphenolic compounds of nuts (Source: Bolling et al., 2011).	25
Figure 5. In 2019, almond production amounts to countries (Source: FAOSTAT, 2019).	27
Figure 6. Almond fruit on almond tree (Source: RawFoodLife, 2019).	28
Figure 7. Almond kernel (seed) with skin (Source: Quora, 2020).....	29
Figure 8. The experimental setup of the water vapor permeability test.....	35
Figure 9. PV determination setup of the oil samples.	36
Figure 10. Experimental setup of the radical scavenging activity test.....	37
Figure 11. Almond skins after blanching (a), powdered almond skins (b), the supernatant from the extraction (c), the extract residue after the solvent evaporation (d).....	39
Figure 12. Calibration curve of gallic acid standard. Error bars represent the standard deviations of recorded absorbance values (n=3).....	40
Figure 13. Solvent casted PLA and PLA-ASE film samples. a ₁ , b ₁ , c ₁ , d ₁ are films inside the mold. a ₂ , b ₂ , c ₂ , d ₂ are self-standing films.....	41
Figure 14. UV-Vis spectra of PLA and PLA-ASE film samples.....	42
Figure 15. Transparency values of PLA and PLA-ASE film samples. Error bars represent the standard deviations of transparency values (n=3).	43
Figure 16. FT-IR spectra of PLA, PLA-ASE film samples, powdered, and solution of ASE. Labels are the characteristic peaks of ASE.	46
Figure 17. WVP values of neat PLA and PLA-ASE film samples. The different superscripts are significantly different (p<0.05). Error bars represent the standard deviations (n=3).	49
Figure 18. Results of the peroxide value assay for the oil samples covered with PLA and PLA-ASE films. Different superscripts are significantly different (p<0.05). Error	

bars represent the standard deviations (n=3).....	50
Figure 19. Antioxidant activity values of neat PLA and PLA-ASE film samples. The different letters on the bars are significantly different(p<0.05). Error bars represent the standard deviations (n=2).....	51
Figure 20. DSC thermogram of neat PLA film sample.	52
Figure 21. DSC thermogram of 1.8 wt.% PLA-ASE film sample.....	53
Figure 22. DSC thermogram of 3.2 wt.% PLA-ASE film sample.....	53
Figure 23. DSC thermogram of 4.6 wt.% PLA-ASE film sample.....	54
Figure 24. SEM surface micrographs of PLA and PLA-ASE films. (a,e) Neat PLA; (b,f) 1.8 wt.% PLA-ASE; (c,g) 3.2 wt.% PLA-ASE; (d,h) 4.6 wt.% PLA-ASE. The left column's magnification: 500x; scale bar: 300 μm , the right column's magnification: 2500x; scale bar: 50 μm	56
Figure 25. RMS values of PLA and PLA-ASE film samples for 5x5 μm^2 . Error bars represent the standard deviations (n=2).	57
Figure 26. 3D AFM images of PLA and PLA-ASE films for area 5x5 μm^2 . Neat PLA, 1.8, 3.2, and 4.6 wt.% PLA-ASE films are a, b, c, d, respectively.	58
Figure 27. 3D AFM images of PLA and PLA-ASE films for area 10x10 μm^2 . Neat PLA, 1.8, 3.2, and 4.6 wt.% PLA-ASE films are a, b, c, d, respectively.....	59

CHAPTER 1: INTRODUCTION

Different types of food wastes arise from processed vegetables and fruits as a result of the activities of various industrial sectors such as agriculture and food (Kumar et al., 2017). In addition to this large amount of wastes in the sectoral market, household, hotel, and restaurant wastes also gather in urban solid waste storage plants. Further, this accumulation, which is open in the open area due to insufficient solid waste management, plays an active role in environmental pollution (Babbar et al., 2013). However, these vegetable and fruit wastes can be potential sources of plant-based bioactive compounds (phytochemicals) (Kumar et al., 2017). One of the most effective roles in waste management is the effective recovery of phytochemicals. These bioactive phytochemicals, which are rich in antioxidant properties, can be used as functional food additives to extend shelf life, as anti-aging product ingredients in the cosmetics industry and many fields such as medicine (Kumar et al., 2017; Babbar et al., 2013). To support the sustainability of agriculture and food systems, isolation of these valuable compounds as natural antioxidants from solid wastes can be among the innovative approaches of the future (Chen et al., 2019).

Besides the natural antioxidants, synthetic antioxidants are also used for various applications. Synthetic antioxidants that are commercially produced from chemical sources do not present sufficiently effective antioxidant activity due to their low solubility. Also, their safety and toxicity issues are the biggest concern (Chen et al., 2019; Barlow et al., 1990). Because of these reasons, interest in researching the use of natural antioxidants increases day by day.

Polyphenolic compounds, a type of phytochemical, are found in vegetables and fruits as secondary metabolites and display antioxidant activity. This activity is also called radical scavenging activity and it supports human health (Meshkini, 2016). Besides, natural antioxidants are also under the spotlight in the pharmaceutical, cosmetic, and most particularly in the food packaging industry to prevent oxidative stress (Moo-Huchin et al., 2015).

Nuts (natural or roasted) and their by-products (such as hull, shell, or skin) are one of the richest sources of natural polyphenolic compounds (Chang et al., 2016). Furthermore, their lower cost and contribution to sustainability can be considered as important factors, therefore, being a research subject of interest in the literature.

Almond (*Prunus amygdalus L.*), which is one of the hard-shelled fruit species and is included in the Rosaceae family, which includes fruits such as apples, pears, and raspberries, is one of the most popular tree nuts in the worldwide nut production order (Esfahlan et al, 2010). *Amygdalus* subgenus comprises 40 species of almond and 12 species of them are grown in Turkey (Soylu, A., 2003). Almond varieties are grown in many regions of our country. However, those grown in Muğla-Datça in the Aegean Region are the best known (Erdem İşmal and Yıldırım, 2012). According to the United Nations Food and Agriculture Organization data, almond production in Turkey in the last decade has increased day by day and settled in 4th place worldwide with an annual production of 150,000 tonnes (FAOSTAT, 2019). Commercially, almonds can be consumed in shell, unshelled, and peeled (separated from the brown skin on the original seed). Especially peeled almonds are widely used in the confectionery and baking industry. In such industrial applications, the method of blanching in hot water is generally used to separate the almonds from their skins and the resulting by-product (skin) is separated as waste (Chen et al., 2019). The byproducts (shells and skins) separated from almonds can produce a large amount of waste. Based upon the high production rates of almonds in Turkey and the world, recovery of these by-products, which are generated as waste from mainly the food industry, has become an important issue.

In the study conducted by Varzakas, Zakynthinos and Verpoort (2016), it was reported that bioactive (antimicrobial/antioxidant) polyphenolic compounds are mostly concentrated in the membranes (skins) and seeds of fruits. According to literature, one of the places where active compounds are most concentrated in almonds has been recorded as skin (Wjeretna et al., 2006). The main polyphenolic compounds of almond skin are flavonols, flavonol glycosides, and non-flavanoids (phenolic acids) (Iva Prgomet et al., 2017). Almond skins, which are by-products, exhibit antioxidant activity in vitro and in vivo by under their high polyphenolic content (Chen et al., 2019). The valuable bioactive compounds obtained from almonds have multi-purpose use potential in various sectors. Therefore almond waste recovery has great importance.

Especially with this waste recovery approach, composite biomaterial studies using natural resources is a current research subject. Almond by-products are also valuable sources for this purpose because of their structures and content of bioactive

compounds. With its cellulosic structure and high fiber content, the almond peel has been used as a supporting (reinforcing) additive for polymer matrices in biocomposite material studies. In previous studies, the composite material which is prepared with various compatibilizers by using almond shells as flour (fine powder) or in the form of small particles, showed better physicochemical properties compared to the pure (non-additive) polymer (Quiles-Carrillo et al., 2018; Zahedi et al., 2015; Esssabir et al., 2013). In addition to contributing to the mechanical properties of the polymer such as durability, it also positively affects environmentally friendly degradation (biodegradability). Therefore it has been an interesting research subject for use in the plastic industry (Liminana et al., 2018). Similarly, almond skin which is another by-product also studied with polymers such as polyamide6 (PA6), poly(lactic acid) (PLA), poly(ϵ -caprolactone) (PCL). The studies aimed to improve mechanical properties, to investigate changes in thermal stability and barrier properties of the polymer (Valdés et al., 2016; Mankotia, Singh and Singh, 2020; Singh et al., 2020). Almond skin was used in powder form and its promising reinforcement effect was reported. Additionally, its effect on thermal stability varied according to polymer type and also almond skin concentration. The higher water absorption was observed in the polymer materials including almond skin powder because of the hydrophilicity of the almond skin content.

A research study of composite polymer film using almond skin extract (ASE) with PCL has been made by García et al. (2020). They reported that ASE had no significant effect on the structural, thermal, and mechanical properties of the PCL. However, the extract affected the gas barrier properties of the PCL in a positive way and also provided an antioxidant activity to PCL film. They suggested that PCL-ASE films promising material for functional food packaging systems.

According to the best of our knowledge, there is no information about the PLA composite study that has been found in the literature containing almond skin extract.

In this study, PLA was used as a polymer matrix. Particularly, biodegradable and biobased on renewable raw materials bioplastics such as PLA can be the best chosen for the composite biomaterial research. PLA, a member of the aliphatic polyester family, is a high-strength thermoplastic. Lactic acid which is the building block of PLA can be produced by fermentation of sugars obtaining from renewable raw

materials such as sugar beet and corn. For these reasons, PLA is an alternative environmentally friendly plastic material to traditional petroleum-based plastics (Farah et al., 2016).

The present study has three specific objectives; the first objective was the extraction of polyphenolic compounds, which are bioactive compounds with antioxidant properties, from almond skins with high yield. The second objective was the incorporation of ASE into the PLA solution and a solvent casting technique was used for the film-forming. The third objective was to evaluate various properties of PLA-ASE composite films such as structural, mechanical, morphological, optical, thermal, gas barrier, and possible antioxidant properties. The overall aim of this research is to explore the possibility of using PLA-ASE composite films as functional food packaging materials.

The outline of this research thesis is as follows, the structural characteristics of the PLA and its general applications were reviewed in Chapter 2 to understand why it is used and its promising points. In addition to these, information about the almond fruit and its structural components and contents were presented in the same chapter. All of the experimental procedures were defined in Chapter 3, comprising polyphenolic compound extraction from almond skin and its total polyphenolic content determination, development of PLA-ASE composite films, and characterization studies of the film samples to investigate the effect of ASE on the PLA. The results obtained from the experiments and their relations with each other were presented in Chapter 4. After all, remarkable outcomes of the study were presented in Chapter 5 to conclude all findings.

CHAPTER 2: LITERATURE REVIEW

2.1 Poly(lactic acid)

Recently, sustainability is the most important factor for the green environment. To support sustainable development, the management of waste caused by fossil-based plastics is the fundamental issue. Using bio-based polymers (derived from renewable sources) is the best way to overcome this problem. Even definite biobased manufacturing plastics are previously usable in the market, poly(lactic acid) is the most commonly used biobased polymer as an alternative to petrochemical-based polymers. The motivation here is to benefit from the cost-efficiency and distinctive features of PLA (Julien et al., 2012; Nagarajan, Mohanty and Misra, 2016).

The evolution of PLA began with the formulation of lactic acid by Bischoff and Walden in 1893 and then low molecular weight PLA was generated by Carothers and collaborators in 1932. Firstly, PLA marketing was initiated in medical applications such as sutures, drug-delivery systems, and etc. in 1954. In 1992, PLA fibers were produced in the laboratory, then commercial fabrication was started under the commercial name Lactron in 1994, Japan. This was followed by Fiberweb France S.A., France under the name of Deposa in 1997 and then Cargill Dow LLC, the USA as NatureWorks in 2002. In 2005, the trading name was renamed NatureWorks LLC which is the largest producer of PLA (Rasal, Janorkar and Hirt, 2010).

PLA is a member of the aliphatic polyester family. It is a biodegradable thermoplastic, high-strength, and high modulus polymer. The Institutur unit of PLA is the lactic acid. It has two enantiomers as L- and D- lactic acid due to this PLA has stereoisomers; poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and also poly(DL-lactide) (PDLA). Lactic acid can be produced from the fermentation of sugars that are procured from renewable feedstocks such as sugarcane, potatoes, corn, and etc. Therefore, PLA is eco-friendly plastic material instead of traditional petroleum-based plastics (Farah, Anderson and Langer, 2016). Figure 1 represents the life cycle from the production of the PLA back to its recovery.

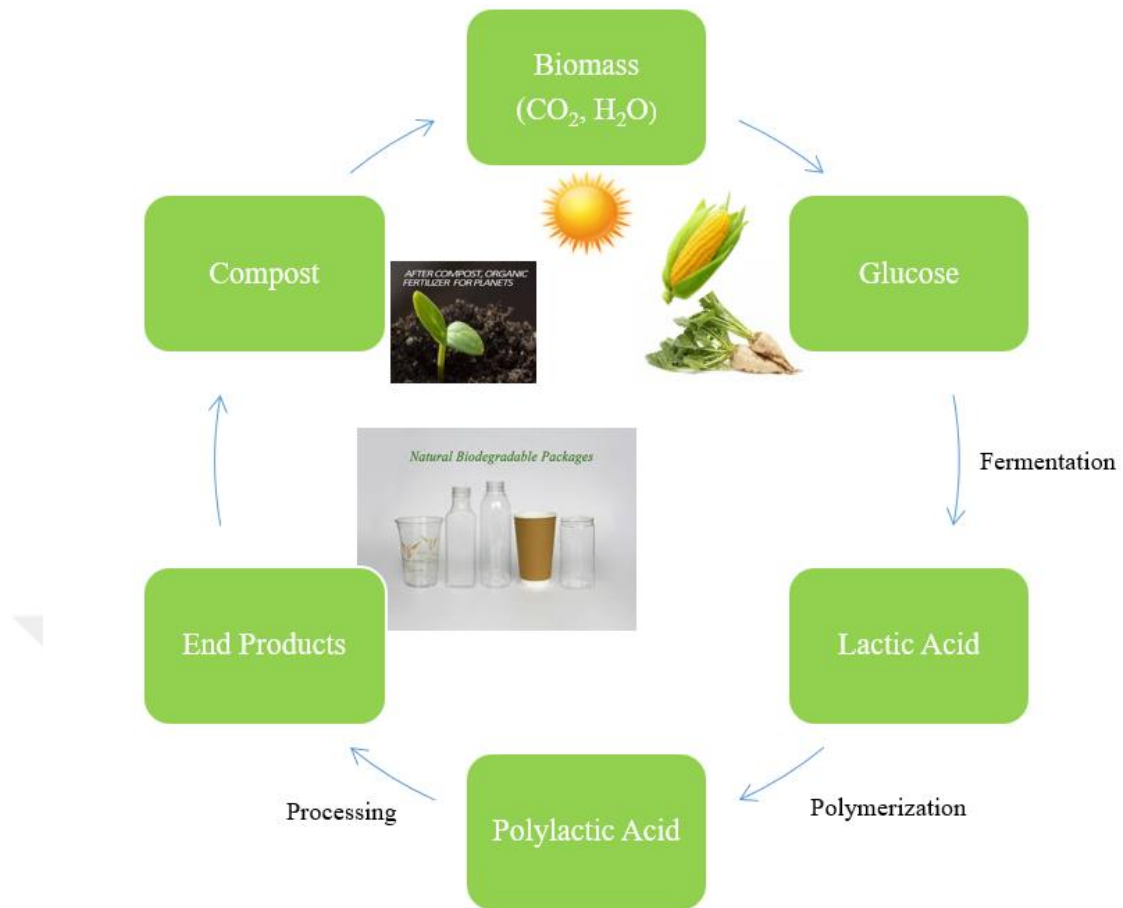


Figure 1. The representative basic life cycle of PLA (Source: Auras, Harte and Selke, 2004a).

2.1.1 From lactic acid to poly(lactic acid)

Lactic acid ($\text{CH}_3\text{-CHOHCOOH}$) which is the fundamental building block of PLA can be produced by fermentation or chemical synthesis (Lim, Auras and Rubino, 2008). A great majority of lactic acid (almost 90%) is produced by microbial fermentation instead of the chemical route. While the chemical process presents disadvantages such as the racemic mixture of lactic acid as a product, fermentative production provides pure L- or D-lactic acid. Also, the fermentation method supports the usage of renewable feedstocks as a source of glucose (Adsul, Varma and Gokhale, 2007). Lactic acid exists in many foods either naturally like corn, potato or as an output of microbial fermentation, as in milk, yogurt, and etc. In addition, lactic acid is a natural product of metabolism of most living organism (Datta and Henry, 2006). It acts on cellular activity, diversely. The important one is an energy substrate for the metabolic functions of cells. Lactate can permeate through the cell membrane because of its small size and uncharged character. After entering the cell, lactic acid is turned

to glucose which is an energy source for the cell cycles. Another substantial issue is the antioxidant function of lactic acid. By way of this property, it can protect cells against damages of free radicals, producing naturally in the life cycle (Lasprilla et al., 2012).

Owing to two functional groups –carboxylic and hydroxyl groups- of lactic acid, it can take place in multifarious chemical reactions. By using chemical reactions such as esterification, condensation lactic acid can be used for many industrial application products such as pharmaceutical, food, textile, and etc. (Datta and Henry, 2006).

Polymers of PLA can be produced by different production pathways (Figure 2). For the industrial production of PLA, ring-opening polymerization (ROP) is the most widespread method (Armentano et al., 2013). This way provides to obtain the high molecular weight of PLA rather than direct polycondensation of lactide. In every step of polycondensation, one water molecule is formed, the existence of water molecule causes degradation of polymer molecule and reaction is concluded with the production of low molecular weight of PLA (Pang et al., 2010). For these reasons, ROP becomes a better alternative for producing PLA. This method also presents an opportunity to obtain pure PLA as optically and crystallinity (Jamshidian et al., 2010). In this process, firstly low molecular mass pre-polymer (mid product) from the monomer is produced. Then it is turned into lactic acid stereoisomers (L-, D- and meso-lactide) under low pressure by condensation reaction. Ring-opening polymerization is initiated after the vacuum distillation of the lactide mixture. This polymerization step can be anionic or cationic regarding the initiator (Auras, Harte and Selke, 2004a). The most common active initiator for the production of L-lactide is stannous octoate (bis 2-ethyl hexanoate, SnOct₂) which is approved by the Food and Drug Administration (FDA) (Piaux et al., 2007).

Also, condensation reactions (direct or azeotropic) are other techniques for the polymerization of lactic acid. As a result of the direct condensation, glassy, brittle, and low molecular weight PLA is released. To overcome the problem of low molecular weight production, certain adjuvants such as esterification and chain-extending agents are used. Since these agents require to use of solvents, extra purification steps occur. This statement causes a cost increase. Besides this, some adjuvants do not completely react and residues that are not biodegradable and bioabsorbable remain in the polymer.

Hereat final polymer quality and application chance decrease (Pang et al., 2010).

Another method of PLA production is azeotropic condensation which is also known as direct condensation in azeotropic solution. This solution which is a mixture of two or more chemical liquids (their ratios can not be changed by distillation) helps to facilitate distillation pressure and PLA fractionation from solvent (Jamshidian et al., 2010). In this route, there is no need to use extra solvents and also removal of water which is formed during the reaction is easier, relatively. It is possible to obtain the high molecular weight of PLA (Lasprilla et al., 2012).

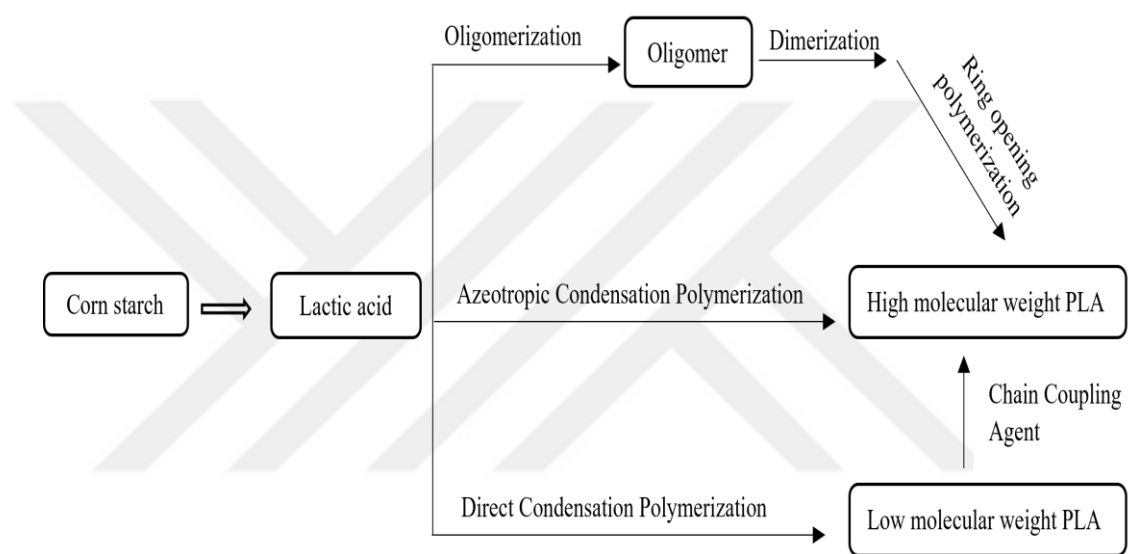


Figure 2. Synthesis routes for PLA (Source: Pang et al., 2010)

2.1.2 Some of the properties of PLA

2.1.2.1 Mechanical properties

High molecular PLA presents good mechanical properties comparable to commercial plastics such as polyethylene terephthalate (PET), polystyrene (PS) (Auras et al., 2003). It is located between two of them and this makes PLA suitable for processing. It has good processability in industrial applications such as containers, cases, and etc. (Carrasco et al., 2010).

Since PLA films have many advantages for use in various areas, mechanical features have been searched by research specialists. Accordingly, PLLA presents better mechanical behavior than other structures' (Perego, Cella and Bastioli, 1996; Garlotta, 2019) and this shows an increment with annealing treatment that is made to get crystallinity. For the pure PLLA, tensile strength values are at around 55-59 MPa.

However, annealed PLLA can reach up to 69 MPa with molecular mass increase (Perego, Cella and Bastioli, 1996).

PLA has naturally good stiffness and high strength. These qualifications are affected by many factors. PLA crystallinity is one of the affecting parameters for mechanical performance as mentioned above. It may lead to developments in strength, stiffness, thermal and chemical resistance (Harris and Ellen C., 2008). Also, stereochemistry and orientation of polymer affect the mechanic behavior (Farah, Anderson and Langer, 2016). Besides these, due to the inherent brittleness of PLA, there can be some limitations related to the production of polymer products. To overcome these drawbacks and improve the features of polymer, there have been many studies on mechanical characteristics.

As one of these research works, according to studies of Lalla and Chugh (1990) and Farah, Anderson and Langer (2016) pure PLA and oriented or different stereochemical composition of PLA shows different mechanic behavior. These changes depend on the degree of orientation and chemical structure. Table 1 lists the results obtained from the mentioned study as a range.

Table 1. Mechanical properties of unoriented and oriented PLA (Source: Lalla and Chugh, 1990; Farah, Anderson and Langer, 2016).

Properties	Unit	Unoriented	Oriented
Tensile strength (TS)	MPa	47.6 – 53.1	47.6 - 166
Elongation at break (EB)	%	3.1 – 5.8	15 - 160
Tensile modulus (TM)	MPa	3447 - 4000	3889 - 4137
Yield strength (YS)	MPa	45.5 – 61.4	N/A

2.1.2.2 Thermal properties

However, PLA has similar mechanical properties to conventional polymers such as PET, PS, it possesses considerably less thermal stability (Pang et al., 2010). For the neat poly(lactic acid, thermal transitions were shown in Table 2. Glass transition temperature (T_g) was recorded from Kulinski and Piorkowska (2005a) and Madhavan Nampoothiri, Nair and John (2010). Melting temperature (T_m) was summarized from

the studies of Auras et al. (2003), Kulinski and Piorowska (2005a), Madhavan Nampoothiri, Nair and John (2010), Pang et al. (2010). The wide range in the T_m is caused by mild racemization or some impureness in the polymer (Pang et al., 2010). Crystallization temperature (T_c) was modified from Henton et al. (2005), Harris and Ellen C. (2008).

Table 2. Thermal transition points of PLA.

Temperature	$^{\circ}\text{C}$
Glass transition (T_g)	50-60
Melting (T_m)	150-180
Crystallization (T_c)	105-115

Structural features of PLA as molecular weights and stereoisomer content can affect the thermal properties (Saeidlou et al., 2012).

T_g is related to the molecular weight of the polymer and this relation was defined by Flory-Fox equation (eq. 1):

$$T_g = T_{g^{\infty}} - K/M \quad (1)$$

where T^{∞} is the glass transition temperature for absolute molecular weight, K is a constant, and M is the molecular weight. This relationship was studied on PLA polymers by Dorgan et al. and the effects of molecular mass and structural composition of the polymer were examined (Dorgan et al., 2005).

T_m mostly depends on the D-isomer content in the polymer. It linearly shows a decrease in the D-lactate content. Pure PLLA has the maximum melting temperature which is around 175-180 $^{\circ}\text{C}$. Another notable factor influencing the melting point is the molecular weight as it affects the glass transition temperature. The simplest way which describes this relationship between the molecular weight of the polymer and melting temperature is the following equation (eq. 2):

$$T_m = T_{m^{\infty}} - A/M \quad (2)$$

with $T_{m^{\infty}} = 181.3^{\circ}\text{C}$ and $A = 1.02 \times 10^5^{\circ}\text{C g/mol}$ and M is the molecular weight of polymer (Saeidlou et al., 2012).

Crystallinity is another thermal characteristic. Natural lactides that are derived from the agricultural feedstocks are usually in the L-form and they show crystallinity. The content of the D-isomer unit is a negative effect on crystalline behavior. Besides these, crystallinity also depends on the thermal history of polymer and additives. Crystallization temperature is measured by DSC and may differ by stereoisomer pattern in the polymer (Ahmed and Varshney, 2011). It can be calculated by following equation (eq. 3):

$$X_c(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_f} \right] \times 100 \quad (3)$$

where ΔH_f is the fusion enthalpy of 100% crystalline PLA which is 93.7 J/g (Auras, Harte and Selke, 2004b; Inkinen et al., 2011).

2.1.2.3 Barrier properties

Since PLA is mostly used in packaging applications, there is a need for a clear understanding of PLA's barrier properties. There have been several studies on this subject in the literature. In general, PLA exhibited similar features compared to conventional polymers. It has similar barrier characteristics to PS but is lower than the PET (Hamad et al., 2015).

For instance, the carbon dioxide (CO₂) permeability of the PLA takes place between the PET and PS. PET has the lowest CO₂ permeability coefficient. Under the same conditions (at 0% RH, at 25 °C) the permeation of oxygen has almost ten times lower values than the CO₂ permeability coefficient (Auras et al., 2003). Besides these, permeations of gases of methane (CH_{4(g)}) and nitrogen (N_{2(g)}) which are also important parameters for the packaging were analyzed by Auras and coworkers (Auras, Harte and Selke, 2004b; Auras, Singh and Singh, 2005). And they reported that PLA also showed an intermediate polymer in this regard. Accordingly, the comparison of PLA to other traditional polymers is as in Figure 3.

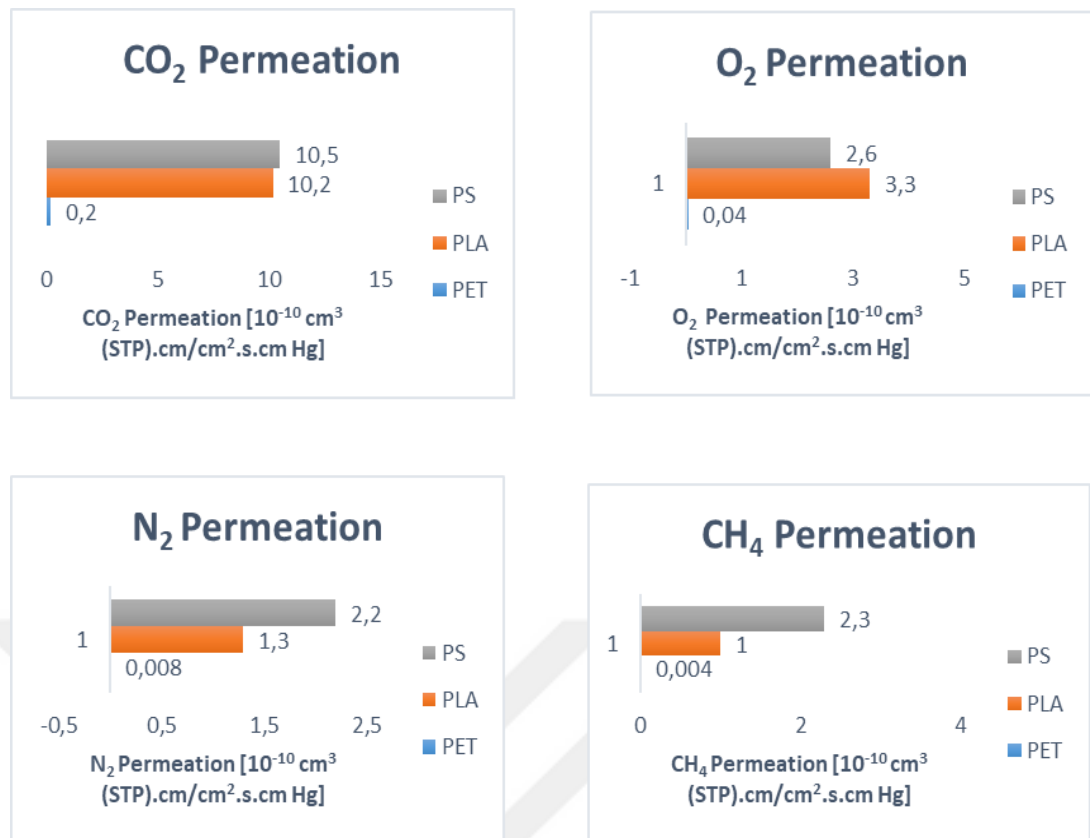


Figure 3. Comparison of gas permeation coefficients at 30 °C (Source: Hamad et al., 2015).

Barrier properties are also affected by many parameters as well as mechanical and thermal properties. In the literature, there are many works about the improvement of the PLA's barrier properties. Researchers have used different additives such as several inorganic and organic compounds, and etc. Thellen and coworkers reported that montmorillonite-layered silicate/PLA composites showed higher oxygen barrier properties (Thellen et al., 2005).

Besides, the effects of synthetic polyphenolic antioxidants on the PLA were investigated by Jamshidian, Arab Tehrani, et al. (2012). They examined that thermal and mechanical properties were affected but antioxidants had no effect on the water vapor barrier properties.

2.1.2.5 Optical properties

As it is well known, PLA has a growing interest as a packaging material in the food industry. For the right design of the package, the transmission of light that affects the food quality such as flavor, taste, shelf life is an essential consideration. Accordingly, the packaging material is tried to be produced in a way to minimize product damage.

Therefore, the optical properties of PLA in other words absorption of visible and UV light by polymer need to be well understood (Auras, Harte and Selke, 2004a).

The electromagnetic spectrum that is related to the packaging applications has three subclasses: Ultraviolet (UV) band (100-400 nm), visible spectrum (400-700 nm), and near-infrared band (700-2200 nm). In this spectrum, absorption and transmission of ultraviolet light are critical to specify the character of polymer (Auras, Harte and Selke, 2004a). UV light spectra also are subdivided into three components: UV-A (400-315 nm), UV-B (315-280 nm), and UV-C (280-100 nm). UV-A has the lowest energy and comprises the greatest part of natural UV-light while UV-C never reaches the earth's surface. As for UV-B, it is the most energetic one and it damages plastics via photochemical degradation.

Transparent PLA film exhibits a high transmission rate in the visible region (400-700 nm). In addition to this, the percent transmittance of light increases in the range of 250-400 nm. This transmittance rate reaches around 95% of UV light at 300 nm which is the undesired circumstance (Arrieta et al., 2014). UV light barrier is an important parameter for the polymer film which is used as a packaging material. It is a significant factor that should not be ignored for the protection of the quality of light-sensitive products during storage (Yang et al., 2016). Therefore, many searches to block transitions in the UV-A and UV-B regions are carried out by scientists.

2.1.3 Major applications of PLA

PLA has become one of the most innovative materials as a biomaterial of recent times. It appears in a key position in the biopolymer market with a wide range of uses, going from biomedical, automotive, textile, to packaging (Table 3). In recent years, new techniques make possible budget-friendly production of high molecular weight PLA. Therefore its utilization is growing (Murariu and Dubois, 2016). Additionally, due to the low environmental footprint of PLA, it has considerable interest for a wide range of industrial applications (Gupta, Revagade and Hilborn, 2007). These applications can be subdivided into two groups; durable consumable goods are commodities having a lifetime of more than 3 years and nondurable consumable goods are commodities with a lifetime up to 3 years. For instance, while packaging materials, short-term medical staff are in the category of consumer nondurable products, car furniture is in the group of durable consumer products (Castro-Aguirre et al., 2016).

Table 3. For the PLA, some commercialized products and industrial application fields
(Source: Jamshidian et al., 2010).

Available Applications	Commercial Products
<i>Plastic Applications</i>	
Rigid thermoforms	Fresh fruit and vegetable clamshells Opaque dairy (yogurt) cups Bakery, biscuit, meat trays, and films Trays or bowls for fast food (McDonald's) Electronics packaging (Panasonic, Sony)
Biaxially oriented films	Floral wraps Shrink sleeves Envelope and display carton windows (Mitsui, Ecocard) Product overwraps (gift packaging)
Bottles	Edible oils Milk containers Bottled waters
<i>Fiber Applications</i>	
Apparel	Sports, casual outfit items (Cargill Dow, Kanebo Gosen)
Nonwovens	Wipes Hygiene products Automotive head and door lines
Furnishings	Blankets (Ingeo) Upholster Wall covering
Industrial Carpets	Agricultural – Mulching films Carpet tiles Landscape drains
Fiber fills	Pillows, comforters Housewares, trash, and vacuum bags

2.1.3.1 Medical industry

The sensitivity of lactic acid which is a monomeric form of PLA to water is an advantage for medical applications. Degradation of medical materials such as medical sutures, pharmaceutical delivery vehicles, and etc. is easier in the human body. In addition to this, degradation products can be excreted easily via kidneys or can be metabolized into the form of water and carbon dioxide (P. Pawar et al., 2014).

Lactic acid is naturally situated in the human body. Therefore, it is harmless to humans. Since PLA can be degraded without any intervention, it is mostly used as medical implants such as bone grafting, fracture fixation appliances, and etc. There is no extra requirement for the removal of them. The replacement of metal tools of medical devices with PLA materials has been a lot of research in recent years. Also when compared with metallic implants, PLA has no toxic effect. Metallic parts can create possible problems for example abrasion or decomposition of magnetic images. To obviate this trouble, PLA is an important alternative. For the development of mechanical properties and functionality of PLA, it is usually combined with different polymers or proteins like PGA (polyglycolic acid), collagen, carbon fiber, and etc. In this way, stabilization of it is increased for the fixation (Castro-Aguirre et al., 2016).

Thus, due to the bioresorbable and biocompatible properties of PLA also the no toxicity of degradation end products, it is highly favored for medical sutures, pharmaceutical delivery systems, and other biomedical applications (Rasal, Janorkar and Hirt, 2010).

The first research about PLA as medical devices was reported by Kulkarni et al. (1971) and it was the first step for further studies. Many types of research are focusing on medical applications of PLA and its composites such as orthopedics, drug carriers, stents, tissue engineering, anti-agents in the literature (P. Pawar et al., 2014).

A growing interest in bio-absorbable orthopedic devices such as tissue growth implants and fracture fixation devices is caused by examination of absorbable materials like PLA. They are generally used for bone grafting, repairing of tendons, treatment of fractures, and etc. Biopolymers such as PLA decrease the infection risk and also promote the fixation and repair process (Athanasίου, 1996). Different composites of PLA exhibit varied facilities for application fields. For instance, calcium phosphate is a common reinforcement for PLA matrix as increasing the degradation

rate in bone surgery (P. Pawar et al., 2014). Another composite example is PLA/chitosan, it is a durable material for a longer time as the remaining strength and shape of the tissue and also considerably neutralizes PLA degradation acidity (Li, Ding and Zhou, 2004). There are many similar studies in the literature and researches is still ongoing.

In the pharmaceutical area, controlled drug delivery systems have a remarkable interest. For this purpose, biocompatibility and biodegradability are vital selection criteria. Also, monomer composition polymer, manufacturing technique, Physico-chemical features, and like that are impact factors, too (Khang, 2013). Among the using biodegradable polymers, PLA is one of the prominent polymers due to its good physical-chemical properties, good biocompatibility, and easy processing. In this field, chitosan is a particularly effective adjuvant for the PLA matrix for composites. It has a positive effect on the release of antigen which is an effective factor for possible vaccines (Tsuji, 2005).

Tissue engineering, a field of medical technology where researches are increasing day by day, is a multidisciplinary science field including engineering, biologic methods, and etc. In this field, solutions are examined for the improvement of biological functions or the replacement of lost organs. There is a required biocompatible tissue scaffold which is polymer support for cell proliferation. PLA composites exhibit effective scaffold performance in terms of good mechanical performance, biologic response, and no toxicity (P. Pawar et al., 2014).

Due to the safety and processability of PLA, it is an interesting material in medical biotechnology for the designing and using of implants and various biologic treatment and improvement techniques.

2.1.3.2 Food industry

Food technologies are one of the most uses of PLA. As a food ingredient, lactic acid is defined as a nonvolatile, inodorous, and GRAS (generally recognized as safe) matter by the FDA. In a diverse range of processed food such as candy, bakery, and dairy products, soft drinks, and etc., it is used as a pH buffering agent, acidulant, flavoring agent, or preventive for bacterial deterioration (Datta et al., 1995). Besides being used as an additive, PLA is a very interesting polymer as a packaging material. Since PLA has essential structural properties like mechanical and barrier for the applications to

vie with conventional (petroleum-based) thermoplastics (Lim, Auras and Rubino, 2008). Due to thermoplasticity, it supplies lots of necessities for packaging applications. It can be easily shaped according to the desired function.

Packaging material must provide longer shelf life to the product inhibiting its spoilage by physicochemical or biological factors. Also, it should be biodegraded within a reasonable time to prohibit environmental waste problems (Armentano et al., 2013). Considering these, presenting advantages of PLA can be listed as in Chapter 2.1.3.4.

2.1.3.3 Other application areas

Production of fiber form of PLA is one of the greatest application areas of PLA. Structure of PLA appropriate for lots of fiber application designing. Some of these are bulk filaments for carpet, filament and spun yarns, hygiene products, nappies, and etc. (Farah, Anderson and Langer, 2016). For instance, since PLA is a naturally polar polymer, it can easily absorb moisture and this makes it a suitable material for wipes. Polymer fiber form is obtained by spinning and it is used for required applications such as wipe production.

In addition to these, polymer fibers are also used in the automotive industry because some compartments are made of plastic. Several automotive corporations prefer to use eco-friendly plastic materials like PLA for interior parts of vehicles such as floor mats, car upholstery. Although the use of biopolymers is of growing interest to the automotive sector, there is required to solve some impediments such as moisture effects, degradation process, and etc. Even PLA had comparable properties to PET for automotive fabrics, it needs improvement to pass abrasion and flammability tests. Before PLA can replace petroleum-based plastics, some requirements must be fulfilled (Castro-Aguirre et al., 2016).

In the textile industry, It can be converted into filament form and used in many textile supplies (Gupta, Revagade and Hilborn, 2007). It is a considering candidate to replace with synthetic polymers like nylon. Besides being an environment-friendly material, the breathability and wicking properties of PLA make it a promising alternative. Because of these outstanding features, PLA becomes a comfortable material for textile fields such as apparel or homeware manufacturing. Also, according to tests which were made for textile applications it exhibited suitable performance with standards of the American Association of Textile Chemists and Colorists (AATCC). However, there

are some limitations for PLA textiles such as the temperature of pressing and ironing, conditions of dyeing and finishing processes. In conclusion, PLA is appropriate to support material for textile applications but it is needed to be developed to compete with traditional petroleum-based polymers (Farrington et al., 2005).

Another application area of PLA is water-based paints. Fujitsu Laboratories Ltd. is a company that worked in this field and their purpose was to decrease the number of volatile compounds in new products to traditional solvent-based products. In addition to this, the same company collaborated with Toray Industries, Inc. In 2005, they produced heat resistance and flame retardant PLA alloy to use in notebook models (Castro-Aguirre et al., 2016).

As mentioned in this chapter and previous chapters, PLA has many different application areas. It is good supporting material for existing technologies and also it takes place in newly developing technologies. For these achievements, many kinds of research are ongoing about the physicochemical, mechanical, and morphological properties of PLA.

2.1.3.4 Advantages and disadvantages of using PLA

The use of PLA has many advantages as compared with traditional petroleum-based plastics. These advantages are listed as follows;

- i. Decreasing of carbon footprint; Production of bioplastics such as PLA consumes carbon dioxide so emits less CO₂ to nature (Dorgan et al., 2001).
- ii. Eco-friendly; In addition to being derived from renewable agricultural sources, PLA is also biodegradable and compostable thus the amount of toxic waste reduces. For sustainability and a green environment, these properties are attractive (Henton et al., 2005).
- iii. Biocompatibility; This is the most challenging issue for the applications, particularly in relation to biomedical and food packaging fields. Since its monomer is lactic acid which is obtained by renewable agricultural feedstocks, material developing from PLA does not leak any toxic residue (Armentano et al., 2013).
- iv. Power economy; For PLA manufacturing, less energy is required than conventional petrochemical-based plastics. Using less energy makes it

avored with regard to cost as well (Armentano et al., 2013; Www et al., 2014; Farah, Anderson and Langer, 2016).

- v. **Processability:** PLA has quite better thermal processability as regards other biopolymers i.e. poly(γ -caprolactone) (PCL), poly(ethylene glycol) (PEG), and etc. The major processing patterns of PLA are based upon melt processing. Typically, trading PLA can be processed with a traditional twin-screw extruder and using melt viscosities. Indeed, NatureWorks procures various grades of PLA for a variety of processing such as extrusion, injection, thermoforming, and etc. (Auras, Harte and Selke, 2004a; Jamshidian et al., 2010).
- vi. **Degradation:** PLA can degrade in the biological environment such as compost. Degradation consists of two fundamental states that are hydrolytic and enzymatic degradation steps. While the hydrolytic stage occurs in bulk polymer through water diffusion, the second stage occurs in polymer surface by microorganisms such as fungi and bacteria. Firstly, water diffuses into the polymer and non-enzymatic chain scission occurs. After that molecular weight of PLA decreases and oligomers and lactic acid monomers which can be digested by microorganisms are formed. Finally, the polymer is metabolized and converted to carbon dioxide, water, and humus (Armentano et al., 2013).

Although PLA has excellent advantages as mentioned above, it has also some limiting drawbacks for particular applications. These disadvantages;

- i. **Poor toughness;** PLA has comparable tensile strength and elastic modulus to poly(ethylene terephthalate) (PET) but it is a structurally brittle material. This brittle characteristic is a limiting factor for its implementations (Auras, Harte and Selke, 2004a; Rasal, Janorkar and Hirt, 2010).
- ii. **Slow degradation rate;** Rate of degradation depends on crystallinity, molecular weight, and morphology of polymer and degradation occurs by way of hydrolysis of backbone ester groups. Degradation rate is a vital criterion for biomedical applications. In some cases, the long in vivo life of polymeric medical material is an unexpected situation. Besides, the slow degradation rate is a restricted problem for the annihilation of consumer

products as well (Janorkar, Metters and Hirt, 2004; Tokiwa and Calabia, 2006).

- iii. Deficiency of reactive side-chain groups; Since PLA is a chemically inert substance, its surface modifications are compelling research issues (Rasal, Janorkar and Hirt, 2010).

To overcome these limitations, there is much research in the literature such as natural or synthetic additives to polymer, blending with other polymers, plasticizers, and etc. (Martin and Avérous, 2001; Yu, Dean and Li, 2006; Rasal, Janorkar and Hirt, 2010; Manzanarez-López et al., 2011; Darie-Niță et al., 2018).

2.2 Poly(lactic acid) composites

As a mentioned previous chapter, PLA has great potential as an alternative to petrochemical-based plastics in application areas such as packaging, textile industry, and etc. The new PLA-based products are developed to fulfill the necessities of specific application areas. Due to the improvement of the performance of PLA material, and also enhancement of versatility, there has been growing interest in composite materials. These improvements may be organic or inorganic fillers for instance talc, clay, graphene, plant-based extracts, fibers, and etc. Another improvement method may be blending with different polymers. Production of these PLA-based composites can be performed by similar manufacturing techniques as in the case of PLA production (Murariu and Dubois, 2016). For the manufacturing process, conditions are specified considering the characteristic of additive and desired end-use features. At the end of the process, PLA acquires different particular end-use characteristics, such as toughness (Anderson, Schreck and Hillmyer, 2008; Liu and Zhang, 2011), anti-UV, antimicrobial properties (Murariu et al., 2011; Huang et al., 2015), flame retardancy (Bourbigot and Fontaine, 2010; Bocchini and Camino, 2012), and etc., depending on modifications and reinforcements. These final characteristics of the composite materials vary by interference between polymer matrix and additive, mixing ratio of blend elements, and also chemical structures of main subjects.

2.2.1 Reinforced PLA with fibers and fillers

Firstly, using natural additives has priority here as well for the green environment, and also another impulsion is low-cost with respect to synthetic additives. There are a lot of researches to develop bio-based composite materials with natural fibers and fillers

(Mukherjee and Kao, 2011; Faruk et al., 2012; Pickering, Efendy and Le, 2016).

Natural fibers such as coconut, cotton, kenaf, and etc. Have favored stiffness and strength for this reason their utilization for composites is a concerning issue for many scientists working on polymer materials (John and Thomas, 2008; Graupner, Herrmann and Müssig, 2009; Mukherjee and Kao, 2011; Faruk et al., 2012; Ramamoorthy, Skrifvars and Persson, 2015; Nagarajan, Mohanty and Misra, 2016). Especially, they contribute to the mechanical performance of polymer matrix. Mechanical features of final composite polymer material depend on the amount and characteristic of adding fibers and also natural structures of polymer, compatibility, production process, and etc. (Pickering, Efendy and Le, 2016). Besides the favored mechanical support of fibers, they have some drawbacks and their hydrophilicity is the major one. Since PLA exhibits high sensitivity to high temperature and hydrolysis, maximum dryness is provided for both polymer matrix and adding fiber. In addition to these, great moisture absorption of natural fibers is another negative side that is to be considered for especially transportation, storage, and also the production process (Bismarck et al., 2002; Pickering, Efendy and Le, 2016).

Cellulose is one of the most used fibers based on natural sources and it is found abundantly in nature (Siqueira, Bras and Dufresne, 2010; Murariu and Dubois, 2016). In the literature, several research studies about cellulose-PLA composite materials using different types of cellulose fibers such as micro-fibers, nanowhiskers, nanocrystals, and etc. (Braun, Dorgan and Knauss, 2006; Ambrosio-Martín et al., 2015; Dhar et al., 2015). Due to biodegradability, nontoxicity, cost-benefit, structural fortification of polymer materials which are reinforced with cellulosic fibers, they have drawn attention from many scientists (Fox et al., 2012). In addition to all these high hydrophilic surface structures of cellulose fibers is a major disadvantage as it causes powerless polymer matrix/fiber interactions which lead to reducing mechanical features. However, it is not an obstacle for utilization because of contributes to easy degradation of PLA in the existence of water and moisture (Siqueira, Bras and Dufresne, 2010). Also, this hydrophilic surface characteristic provides many probabilities for chemical alterations for example using silanes (Pilla et al., 2009).

The utilization of fillers, such as talc, clay, hydroxyapatite, carbon nanotubes, graphite derived, and etc., as reinforcement for PLA, is another research area for developing

physical, mechanical, optic, and rheological characteristics of polymer matrices. Several studies are focusing on the addition of organic-inorganic fillers to PLA in the literature (Bordes, Pollet and Avérous, 2009; Raquez et al., 2013; Brzeziński and Biela, 2014; Murariu et al., 2014).

Since talc has great efficiency and unique constitutional characteristics for the development of PLA performance, it is one of the most favorite fillers for producing composite material. In the literature, there is a huge number of papers and patents recommending that talc improves stiffness and barrier properties, accelerates crystallization rate, increases heat resistance of PLA (Harris and Lee, 2008; Lee et al., 2008; Frone et al., 2011; Ouchiar et al., 2015). Although the amount of addition is also a factor in the developing characteristics, talc plays a role as a nucleating agent for the crystallization rate independently from loading quantity (Harris and Lee, 2008; Ouchiar et al., 2015).

Another mainly used fillers and their beneficial contributions for polymer matrices as in the following Table 4.

Table 4. Mostly used fillers for PLA composite material and their effects (Source: Murariu and Dubois, 2016).

Additive Filler	Effect on PLA	Reference
Talc	Nucleating agent effect on crystallization Improvement of mechanical characteristics	(Harris and Lee, 2008; Ouchiar et al., 2015) (Shakoor and Thomas, 2014)
Hydroxyapatite	Supportive effects, different particular end-use features for biomedical applications	(Gültekin et al., 2004; Zhang et al., 2005)
Graphite and its derivatives	Increment of Young's modulus and storage modulus, uplifted crystallization, improved thermal stability	(Fukushima et al., 2010; Murariu et al., 2010)
Carbon nanotubes (CNTs)	Enhancement of mechanical and thermal characteristics, flame retardant property, developed crystallization	(Xu et al., 2011; Raquez et al., 2013; Brzeziński and Biela, 2014)

2.2.2 Incorporation of plant-based polyphenolic compounds into PLA

Polyphenolic compounds are secondary metabolites that are found in fruits and vegetables. Since their radical scavenging activities, they play an important role in human health as an antioxidant. They reduce the risk of cardiovascular disease, cancer or aging, and etc. (Meshkini, 2016). Outside of the biological characteristics, natural antioxidants are also under the spotlight in the pharmaceutical, cosmetics, and most particularly in the food packaging industry by preventing oxidative stress (Moo-Huchin et al., 2015).

Instead of the insertion of additional agents into the food directly, the implication in packaging material presents more advantages such as less toxicity, lower final cost. Natural antioxidants obtaining from food by-products have been growing interest for functional packaging materials due to their biodegradability, non-toxicity, cost-effectiveness, environmental-friendly, and etc. Also, their oxygen scavenging activities are mainly considered active packaging criteria because of preventing or decreasing of oxidation of food (Rai, Dutta and Mehrotra, 2020). In the literature, there are many studies interested in using polyphenolic compounds, as natural antioxidants, obtaining from agro-food wastes or by-products for packaging systems (Valdés et al., 2014; Torres-Giner, Prieto and Lagaron, 2020). Also, there are many types of research using several types of polyphenolic compounds based on the PLA matrix (Table 5). The effects of additives on mechanical, thermal, and physicochemical properties of PLA were analyzed and many different final declarations were offered.

Table 5. Effects of some different polyphenolic extracts that were studied in the literature on PLA film.

Polyphenolic Extract	Characterization Studies of PLA Composite Film	Reference
Olive leaf	Antimicrobial activity: + TS (MPa): ↓ EB (%): ↓	(Erdohan, Çam and Turhan, 2013)
Rosemary	Antimicrobial activity: + Antioxidant activity: + Elastic modulus (EM) (MPa): ↑ TS (MPa): ↑ EB (mm): ↓	(Darie-Niță et al., 2018)
Grapefruit seed	Antimicrobial activity: + TS (MPa): ↓ E (%): ↑ EM (MPa): ↓	(Wang and Rhim, 2016)
α-Tocopherol	Antioxidant activity: + UV Transmission (320 to 260 nm) (%): ↓	(Manzanarez-López et al., 2011)
Tea	Antibacterial activity: + TS (MPa): ↓ E (%): ↓	(Liu et al., 2018)
α-Tocopherol and resveratrol	UV Transmission (350 - 200 nm) (%): ↓ TS (MPa): ↑ EM (MPa): ↑ EB (%): ↑↓	(Hwang et al., 2012)

Nuts (natural or roasted) and their by-products (such as hull, shell, or skin) are one of the richest sources of natural polyphenolic compounds (Chang et al., 2016). Particularly, the skin has the most abundant polyphenolic content in the whole nut (Taş and Gökmen, 2017). There are many different types of polyphenolic components and their subclasses are as shown in Figure 4. Due to the variety of types and complex nature of polyphenolics, their extraction from nuts depends on the structure of the nut and the chemistry of polyphenolic compounds (Shahidi, Alasalvar and Liyana-Pathirana, 2007).

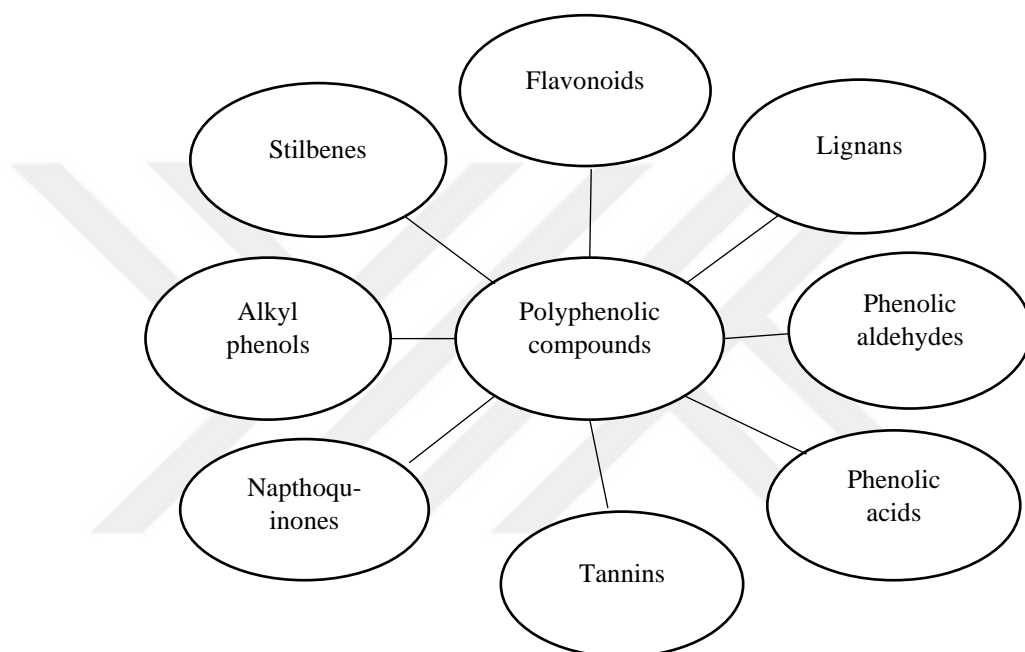


Figure 4. Main subclasses of polyphenolic compounds of nuts (Source: Bolling et al., 2011).

Edible nuts could be processed before consumption or directly consumed as raw (natural). Hazelnut, walnut, pistachio, and almond are the most commonly consumed nuts. While walnuts could be consumed with their skins as natural, other nuts are consumed in a processed form such as roasted, fried, boiled, and etc. as regards preferred end product. For the final form without skin, industrial blanching or peeling stage could be applied (Taş and Gökmen, 2017). After these steps, skins, which have high polyphenolic content, were removed as waste/by-products. Therefore, reused of these valuable by-products has been growing interest for the last years (De Camargo et al., 2014; Özdemir et al., 2014; Bertolino et al., 2015; Smeriglio et al., 2016). As mentioned before, using their extracts or powdered form are also a concerning issue

for the development of polymer materials and this is one of the ways for waste recycling.

2.3 Almond (*Prunus dulcis*)

2.3.1 Almond as a valuable agricultural product

The almond is a member of the Rosaceae family and it belongs to the genus of *Prunus* is located in the *Amygdalus* subgenus, allied with “stone fruits” that have stony endocarps such as peach, cherry, plum, and etc. The almond tree is one of the most popular nut trees around the world because of its robust character against difficult climate conditions such as aridness. Additionally, almond is a nut that has highly nutritive value including lipids, carbohydrates, proteins, various vitamins and minerals, and also polyphenols (Prgomet et al., 2017).

The annual production amount of almonds as a valuable agricultural product is over 3 million tonnes in the world (Kanik and Kabak, 2019). According to FAO statistics, The United States of America (USA) ranks first place with 56.8 percent of global almond production in the last ten years (2009-2019) and it is followed by Spain, Iran. Also, Turkey is placed in the top 10 producers of the world with an annual production of 150,000 tonnes as to data of last ten years (FAOSTAT, 2019). Almond is a kind of fruit that is also consumed in unripe form, known as green almond, in Turkey. In this form, it is an early summer fruit type, and then when it has reached to fully developed and hardened form, it gains more importance in terms of consumption and economy. (Kanik and Kabak, 2019) According to actual data, Turkey has increased the production rate of almonds day by day and it has settled into 4th place in the world (Figure 5) (FAOSTAT, 2019).

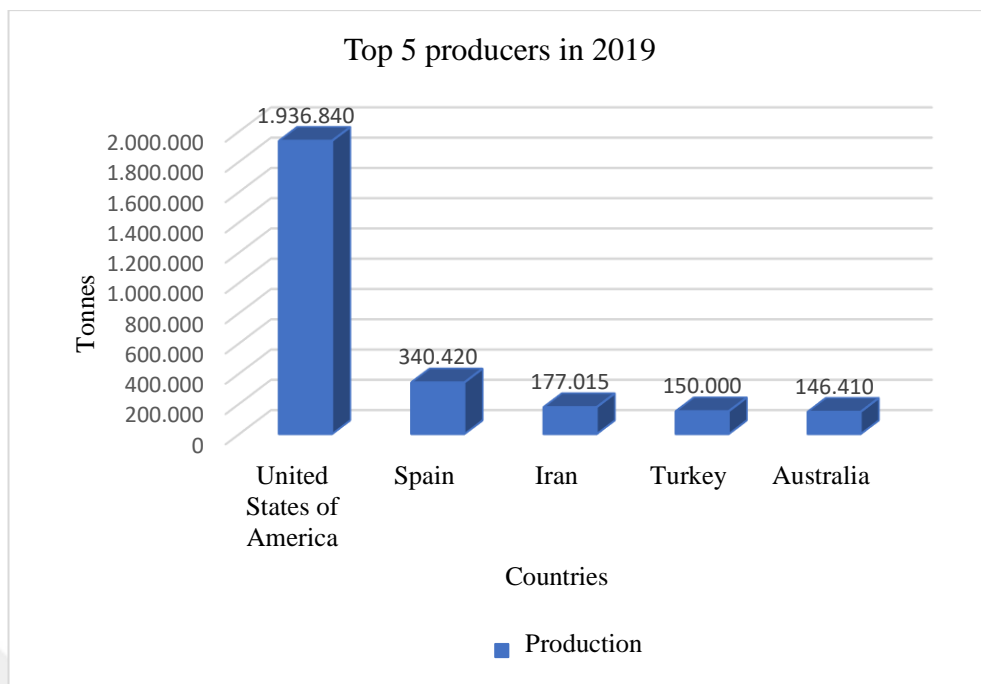


Figure 5. In 2019, almond production amounts to countries (Source: FAOSTAT, 2019).

Since there are many consumption areas of almond and its compatible and resistant structure against different climate conditions also earlier production yield than other shelled fruits, interest in almond cultivation has been increased day by day.

There are two main types of almond fruit depending on tastes as sweet and bitter. Sweet almonds can be consumed directly or used in the food industry without any pre-treatment. However, bitter almonds are not used directly because of having toxicity. Their toxicities arise from cyanogenetic glucosides in their structures. They can be used in the pharmaceutical and cosmetic industry, after the removal of the secondary metabolites that are toxic compounds (Nasirahmadi and Miraei Ashtiani, 2017).

Sweet almonds, mostly consuming ones, are also classified by case (shell) hardness. They are hand, tooth, hard-shelled, and stone almonds, ranked according to their hardness levels of shell from low to high. Among them, hand almonds, having the thinnest shell, have the highest production yield. Also, in most of the almond production regions (ABD, Australia, Argentina) around the world, soft-shelled almond varieties are the most produced variety (Socias I Company, Gómez Aparisi and Alonso, 2005). There are many species of almonds of foreign origin and *Nonpareil*, which is in the group of the California almonds, is the most yielding type (Kuden et

al., 2014).

2.3.2 Structural composition of almond

Almond fruit consists of 4 main parts including hull, shell, kernel, and skin. The kernel, which is the seed of the fruit, is the edible part of almonds. Shell and hull are hardened outer layers covering the seed. During the ripening period, the kernel (seed) is protected by layers against environmental conditions (Gradziel, 2009). The skin, which is the thin layer, is located on the seed and then followed by shell and hull, respectively. The structures of almonds other than the seed are also called by-products. The following Figure 6 shows the structure of whole fresh almond fruit.

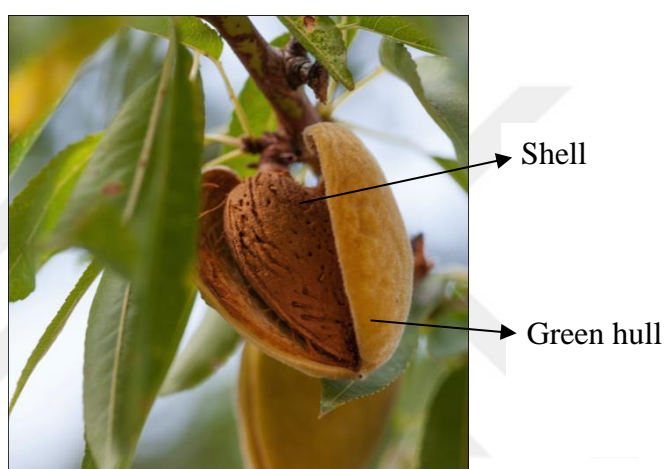


Figure 6. Almond fruit on almond tree (Source: RawFoodLife, 2019).

The large quantities of almond by-products are derived from almond production and processing. While the kernel is most used nutritionally and commercial product, shell, skin, and hull that are by-products remain underrated as a research area. Working on their valuable structural and chemical compositions is essential for significantly recycling them (Prgomet et al., 2017).

During the harvesting or industrial processing, mostly releasing material is the green hull which is around 52% by weight of whole fresh almond. Followed by, the shell is around 33% and kernel (comprising skin) is around 15% by weight of fresh almond. Furthermore, blanching processes, removing skins in industrial processes, causes the formation of additional two by-products as blanching skin and blanching water. Blanched skin constitutes around 4%-8% of the total fresh weight of almonds (Prgomet et al., 2017). In the light of this information, process end products of almond fruit, having bioactivity, comprise 70%-85% by weight of whole fresh almond. A huge

amount of these materials and their valuable compositions make them promising research fields for alternative applications.

Almonds and their by-products (Figure 7) have powerful radical scavenging activities. This power is caused by the phenolic acids and flavonoids which are found in the almond fruit and its by-products (Wijeratne, Amarowicz and Shahidi, 2006). Although almond skin just comprises around 4% of the total almond mass, the almond polyphenolic compounds mostly (60%-80%) are located in the almond skin. Since the total polyphenolic content of the skin, the almond skin was chosen as the richest source of the polyphenolic content of almonds (Wijeratne, Abou-Zaid and Shahidi, 2006; Prgomet et al., 2017).



Figure 7. Almond kernel (seed) with skin (Source: Quora, 2020).

The main polyphenolic compounds of almond skin are flavonols and flavonol glycosides pursued by non-flavanoids (phenolic acids) (Prgomet et al., 2017). Table 6 shows polyphenolic compounds of the almond skin. These polyphenolic compounds exhibit radical scavenging activity. According to previous studies, the highly rich antioxidant activity of almond skin extract was determined by the radical scavenging methods DPPH (Mandalari et al., 2010; Sang et al., 2002; Chen and Blumberg, 2008; Monagas et al., 2009) and ABTS (Smeriglio et al., 2016).

Table 6. Polyphenolic compounds in the almond skin (Source: Prgomet et al., 2017).

Polyphenolic Class	Compound
<i>Phenolic Acids</i>	
Cinnamic acids	Caffeic acid
	Ferulic acid
	Chlorogenic acid

Table 6 (continued). Polyphenolic compounds in the almond skin (Source: Prgomet et al., 2017).

	<i>trans-p</i> -coumaric acid
Benzoic acids	<i>p</i> -hydroxybenzoic acid
	Vanillic acid
	Protocatechuic acid
<i>Flavonoids</i>	
Flavonols	Quercetin Quercetin-3- <i>O</i> -glucoside Quercetin-3- <i>O</i> -galactoside Quercetin-3- <i>O</i> -rhamnoside
	Kaempferol Kaempferol-3- <i>O</i> -rutinoside Kaempferol-3- <i>O</i> -glucoside
	Isorhamnetin Isorhamnetin-3- <i>O</i> -glucoside Isorhamnetin-3- <i>O</i> -rutinoside
	Morin
Flavanones	Naringenin-7- <i>O</i> -glucoside
	Eriodictyol-7- <i>O</i> -glucoside

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

A commercial poly(lactic acid) Ingeo™ 4043D was supplied from Natureworks LLC (Minnetonka, MN, USA). The raw almonds were obtained from a local market in Izmir, Turkey. The following analytical grade reagents were purchased from Sigma Co. (St. Louis, MO): methanol, hydrochloric acid, acetic acid, chloroform, sodium carbonate. Gallic acid, Folin-Ciocalteu's phenol reagent, potassium iodide, sodium thiosulphate, starch were obtained from MERCK (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical reagent was supplied by Alfa Aesar (Thermo Fisher, Kandel, Germany).

3.2 Extraction and characterization of polyphenolic compounds from almond skin

3.2.1 Extraction of polyphenolic compounds from almond skins

Before the extraction, the blanching procedure was applied to remove the skins from the kernels. Blanching and extraction procedure were done based on previous studies by Milbury et al. (2006) and Monagas et al. (2007). Firstly, almond kernels were put in the hot water around 70-73 °C for 6-8 min. Then they were placed in cold water around 10 °C for 2-3 min. Skins were removed by hand. The skins were freeze-dried using a FreeZone Freeze Dryer (Labconco, Kansas City, MO) at -50 °C for 20 hours. After the lyophilization, dried skins were grounded in mortar. The powdered skins were mixed with solvent mixture HCl:H₂O:MeOH (3.7:46.3:50, v/v/v) according to 1:15 (solid: solvent, w/v) ratio and sonicated for 15 min followed by 15 min resting time; sonication was performed one more time for 15 min. After the sonication, the skins were centrifuged (4000 rpm, 10 min., 4 °C). The supernatants were collected, and the procedure was repeated one more time. All the supernatants were combined and placed in the laminar flow hood to evaporate the solvent at room temperature away from the light. After the evaporation, the dried almond skin extracts were placed into the desiccator to protect them from ambient humidity. Before the further analyses, the extract powder was dissolved in methanol and the final concentration of stock solution was prepared as 14 mg/ml.

To investigate the extraction yield, which is a representative mathematical value of the extraction efficiency, the following equation (eq. 4) was used;

$$\text{Yield (\%)} = \frac{\text{weight of dried extract}}{\text{weight of dried almond skin}} \times 100 \quad (4)$$

where g of the dried extract is the remained ASE solid after the solvent evaporation, g of dried almond skin is the powdered almond skin used for the extraction.

3.2.2 Determination of total polyphenolic content (TPC) of almond skin extract

The total polyphenolic content of the ASE was assayed with the Folin-Ciocalteu method as described by Singleton, Orthofe and Lamuela-Raventós (1999). Briefly, 5 ml distilled water, 0.1 ml of polyphenolic extract solution (14 mg/ml), and 0.5 ml of Folin reagent (diluted 10-fold) were mixed. Then the mixture was waited for 8 min. and 0.4 ml of 7.5% sodium carbonate was added and the final volume was adjusted to 10 ml with distilled water. The final reaction mixture was left in the dark for 30 min. and then absorbance values were measured at 760 nm with 2.00 nm of the slit on LAMBDA™ 750 UV/Vis/NIR spectrophotometer (PerkinElmer, California, USA). And also, the same procedure was applied to build up a calibration curve using gallic acid as a standard. To build up the curve, five concentrations of gallic acid were used as 0.025, 0.05, 0.1, 0.25 and 0.5 mg/ml. 0 mg/ml of gallic acid was not measured for the curve. The sample absorbance value was unknown, but it was within the range used in the chart. If the value was less than 0.1, it might be necessary to read the initial point (0:0). In this case, the graph was used as is. Total polyphenol content was expressed as mg/ml gallic acid equivalents (GAE) and then converted to mg GAE/ 1 g of the dry weight of almond skin.

3.2.3 Fourier transform infrared (FT-IR) spectroscopy

Attenuated total reflectance (ATR) spectra were collected using the FT-IR spectrometer (PerkinElmer, California, USA). A small quantity of dried ASE and methanolic solution of ASE were placed on the crystal area of FT-IR -ATR. The spectra were determined in the transmittance mode from 4.000 to 600 cm⁻¹, using 64 scans and 4 cm⁻¹ resolution, and corrected against the background spectrum of air. After the measurement, the crystal area was cleaned with pure acetone to remove the residues.

3.3 Film preparation

Poly(lactic acid) resins were dissolved in chloroform (15%, w/v) and the prepared solution was stored at +4 °C to further steps. Film sample solutions were prepared

according to solid sample amounts of extract and PLA resin. The ASE concentrations in the polymer matrix were prepared based on studies of Hwang et al. (2012). The volume of PLA solution was fixed as 10 ml and different concentrations of extract were added to the polymer solution. The PLA-ASE composite films were prepared in three different concentrations as 1.8, 3.2, and 4.6 g of weight percent (wt.%) PLA. Three replicates were prepared for each concentration. Additionally, neat PLA film without ASE was prepared as a control. Neat PLA and PLA-ASE film samples were prepared by using the solvent casting method. PLA and a certain amount of extract solutions were mixed in a beaker for 5-8 min. and sonicated to remove the bubbles in the solution. The final prepared film solutions were poured into the mold and allowed to dry at ambient conditions.

3.4 Film characterization methods

3.4.1 Optical properties

3.4.1.2 Ultraviolet-Visible (UV-Vis) light barrier properties

The light barrier properties of the prepared films (Figure 8) in the visible and ultraviolet light regions were measured at the wavelength range of 200-800 nm with 2.00 nm of the slit with using a LAMBDA™ 750 UV/Vis/NIR spectrophotometer (PerkinElmer, California, USA). Each film sample was cut into small rectangular pieces and placed on a sample holder. Measurements were made in triplicate for each film sample and recorded as a percent transmittance value. The transparency (T) values of films were computed according to the following equation (eq. 5);

$$T = -\log\left(\frac{T_{600}}{X}\right) \quad (5)$$

where T_{600} is the percent transmittance at 600 nm, X is the film thickness in mm (Han and Floros, 1997).

3.4.1.3 Color

Color parameters of the PLA-ASE film samples were determined according to the Hunter Lab color system. Color values of the films which are a^* , b^* , and L^* were measured with the X-Rite eXact Standard colorimeter (X-Rite Pantone, Grand Rapids, Michigan, USA). Two random measurements were taken on the film surface. Total color differences (ΔE^*) of the PLA-ASE composite films were calculated with respect to neat PLA film using equation 6;

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (6)$$

where Δa^* , Δb^* , ΔL^* are the differences in the a^* , b^* , and L^* values, respectively, between the neat PLA film and PLA-ASE composite films.

3.4.2 Structural analyses (Fourier transform infrared spectroscopy (FT-IR))

Attenuated total reflectance (ATR) spectra were collected using the FT-IR spectrometer (PerkinElmer, California, USA). A small piece of each film sample was placed on the crystal area of FT-IR. The spectra were determined in the transmittance mode from 4.000 to 600 cm^{-1} , using 64 scans and 4 cm^{-1} resolution, and corrected against the background spectrum of air.

3.4.3 Mechanical properties

To determine the mechanical properties of PLA and PLA-ASE composite films, modulus of elasticity, tensile strength, and elongation at break were measured using a texture analyzer, TA.XT *plusC*, (Stable Micro Systems, Surrey, UK) according to ASTM D882 standard method (ASTM-D882, 1997). Film samples, waiting in the desiccator at room temperature, were cut into small strips, and they were tested at a crosshead speed of 10 mm/sec with 50 mm initial grip separation.

3.4.4 Gas barrier properties

3.4.4.1 Water vapor permeability (WVP)

WVP values were calculated based on previous studies (Motedayen, Khodaiyan and Salehi, 2013; Saricaoglu et al., 2018; Moghadam et al., 2020) with some modifications. Firstly, the film specimens were cut into suitable sizes ($2 \times 2 \text{ cm}^2$) and glued onto pre-drilled holes in the jar lids. Then, the jars were filled with silica gel to provide 0% relative humidity and the jar lids were tightly closed in jars. Finally, jars were transferred in a desiccator containing distilled water to provide 100% relative humidity and stored at $25 \pm 2 \text{ }^\circ\text{C}$ (Figure 8). The weight changes of jars were periodically recorded as a function of time every 1 h during the first 8 hrs and then every 24 hrs during six days. The test was done in triplicate for each film sample. The WVP ($\text{gm}^{-1} \text{ h}^{-1} \text{ Pa}^{-1}$) values of the films were calculated as the following equation (eq. 7);

$$WVP = \frac{\Delta W * x}{t * A * \Delta P} \quad (7)$$

where ΔW is the weight change of jar (g), x is the average film thickness (m), t is the time (h), A is the exposed film area (m^2), ΔP is the water vapor pressure difference

across the two sides of the film (Pa).



Figure 8. The experimental setup of the water vapor permeability test.

3.4.4.2 Oxygen permeability

There is a relationship between the peroxide value (PV) of oil samples stored inside the film samples and the oxygen permeability of the films. Measurement of the PV of the oil samples is the relative indication of oxygen permeability of the film samples. It is a colorimetric method depending on sodium thiosulphate titration and based on American Oil Chemists' Society Official (AOCS) Method Cd 8-53 (AOCS,1997; Crowe and White, 2001). The test system (Figure 9) was established by modifying previous studies in the literature (Bonilla et al., 2014; Kurt and Kahyaoglu, 2014). Briefly, glass test tubes were filled with around 10 ml of sunflower oil. The mouths of the tubes were covered with film samples and sealed using teflon tapes. In addition, an open-mouth tube filled with oil was added to the system for control. Then, the samples were incubated for 10 days at 60 °C. After the incubation was completed, the oil samples were titrated to measure peroxide values. Determination of peroxide values was performed according to AOCS standard method. Briefly, 5 g of each sample was first dissolved in 30 ml acetic acid: chloroform solution (3/2, v/v). Then, 0.5 ml of oversaturated KI solution was added and shaken for 1 min and 30 ml of distilled water was added. And then, 0.5 ml of 1% starch solution was added as an indicator. Finally, the mixture was titrated with Na₂SO₃ solution until the blue-purple color just disappeared. The volume of sodium thiosulphate solution spent for each titration was recorded for PV calculation. The same procedure also was applied for the blank without sample. PV value was expressed in milliequivalents peroxide/1000 g of oil (meq/kg) unit and calculated as follows (eq. 8):

$$\text{Peroxide value } \left(\frac{\text{meq}}{\text{kg}} \right) = \frac{(S - B) \times M \times 1000}{\text{g of test sample}} \quad (8)$$

where S is the ml of Na₂SO₃ for sample titration, B is the ml of Na₂SO₃ for blank titration, M is the molarity of Na₂SO₃ solution.



Figure 9. PV determination setup of the oil samples.

3.4.5 Radical scavenging activity (RSA)

The antioxidant activity of the film samples was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method, based on previous studies (Jongjareonrak et al., 2008; Norajit, Kim and Ryu, 2010; Fasihnia et al., 2020) with some modifications. Film samples were cut into small pieces of 0.1 g and transferred to falcon tubes. Then, 2 ml of MeOH solution was added to each sample. After that, they were mixed and incubated for 2 hrs (shaker, IKA KS 4000 ic control, Deutschland, Germany) at room temperature. After the incubation, falcon tubes were placed in a centrifuge (Combi-514R, Hanil Scientific Inc., Gimpo 10136, Rep. of Korea) and centrifuged at 3000 rpm for 20 min. The methanolic aliquots of 0.5 ml from each sample were taken into glass tubes and 2 ml of 0.1mM DPPH solution (in methanol) was added then incubated at room temperature in the dark for 30 min. The same procedure was applied for the control using methanol. In Figure 10, part a shows the film samples which were left in methanol on the shaking incubator and part b shows color changes after adding DPPH to methanolic solutions. The absorbance values of the solutions were measured against a blank (methanol) at 517 nm using a spectrophotometer. The percent RSA was calculated as follows (eq. 9);

$$RSA (\%) = \left[\frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \right] \times 100 \quad (9)$$

where Abs_{control} is the absorbance of the control and Abs_{sample} is the absorbance of the

sample (Norajit, Kim and Ryu, 2010).

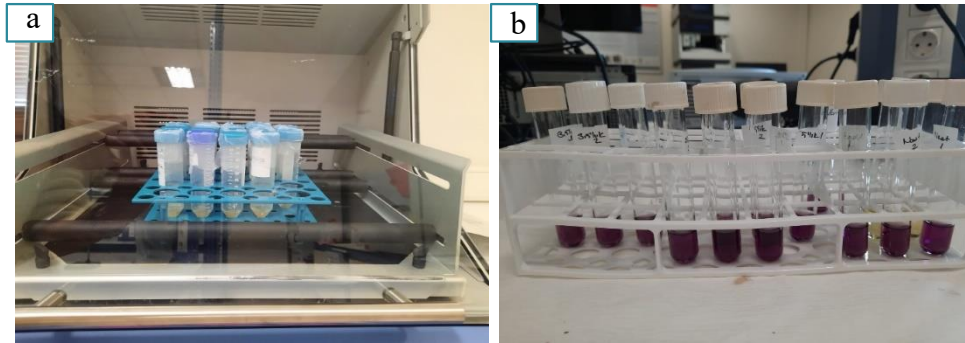


Figure 10. Experimental setup of the radical scavenging activity test.

3.4.6 Thermal properties

The effects of the ASE extract on the thermal properties of the film samples were evaluated using differential scanning calorimetry (DSC) (DSC 4000, Perkin Elmer, California, USA). PLA and PLA-ASE film samples (3-4 mg) were encapsulated in an aluminum pan and then heated from 0 °C to 180 °C, held for 2 min at 180 °C, and then cooled down to 25 °C with a cooling/heating rate of 10 °C/min. under constant nitrogen flow (20 ml/min). The experiment was performed as duplicate. The glass transition temperature (T_g), melting temperature (T_m), and enthalpies of cold crystallization (ΔH_{cc}) and melting (ΔH_m) were determined from the DSC thermograms. The crystallinity degrees (X_c , %) of neat PLA and PLA-ASE composite film samples were calculated using the following equation (eq. 10);

$$X_c(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_f} \right] \times 100 \quad (10)$$

where ΔH_f is the fusion enthalpy of 100% crystalline PLA which is 93.7 J/g (Auras, Harte and Selke, 2004b; Inkinen et al., 2011).

3.4.7 Morphological analyses

3.4.7.1 Scanning electron microscopy (SEM)

The surface properties of the films were analyzed by scanning electron microscopy (SEM, Quanta FEG-250, Zaragoza, Spain). Before the test, film samples (1x1 cm²) were incubated in an oven for 1 h at 60 °C to remove the possible moisture inside the film. Neat PLA and PLA-ASE film samples were sputter-coated with a thin layer of 10 nm gold using an Emitech K550X sputter coater (Labtech, East Sussex, England) before the scanning. SEM images were recorded at high vacuum and 5kV.

3.4.7.2 Atomic force microscopy (AFM)

Atomic force microscopy (AFM) (AFM5000II, Hitachi, Tokyo, Japan) was used to examine surface morphology and measure the roughness index of the polymer film sample. For all film samples, AFM images were recorded at ambient conditions by Multi75AI-G tip with a spring constant of 3 N/m. The tapping mode was used for the whole experiment with scan sizes of 10x10 μm^2 and 5x5 μm^2 . The scan rate was adjusted to 0.5 kHz.

3.4.8 Statistical analysis

All statistical analyses of the experimental data of the PLA and PLA-ASE film samples were determined using the InfoStat statistical software (InfoStat, Version 2012, Cordoba, Canada). A one-way analysis of variance (ANOVA) with the Tukey test model was used to determine the significant differences among the experimental results ($p < 0.05$).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Extraction and determination of polyphenolics from almond skin

4.1.1 Polyphenolic compound extraction from almond skin

Polyphenolic content extraction from almond skin was made with the ultrasonic-assisted solvent extraction method. The dried and powdered almond skins, ASE, and dried ASE as shown in Figure 11.

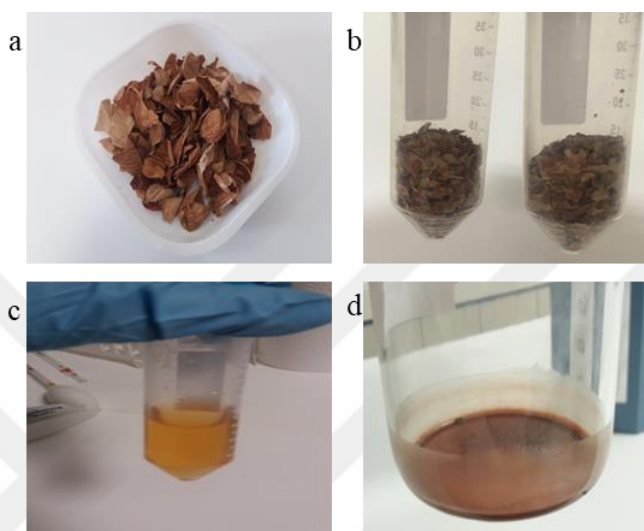


Figure 11. Almond skins after blanching (a), powdered almond skins (b), the supernatant from the extraction (c), the extract residue after the solvent evaporation (d).

For the extraction, 8 g of the dried and powdered almond skin was used and 1.5 g of dried ASE was obtained after the evaporation of the solvent. The value of weighted ASE was converted into percentages. The result of the calculation was 18.75% as the extraction yield. The diversity of almonds and extraction methods leads to variations in yield. In industrial processing, it has been observed that the yield decreases up to 4% in industrial processes (Garrido et al., 2008a).

4.1.2 Total polyphenol content (TPC) of almond skin extract

Folin-Ciocalteu method, a colorimetric method, was used to determine the total polyphenolic content in the almond skin as described by (Singleton, Orthofer and Lamuela-Raventós, 1999). The polyphenolic content was expressed as milligrams of gallic acid equivalent in 1 g of the dry weight of skin (mg GAE/1g dw). TPC was calculated from the calibration curve using gallic acid as a standard (Figure 12). The detailed calculations were presented in Appendix A. The obtained total polyphenolic

content was found as 6.53 ± 0.07 mg GAE/1g dw. This amount is close to results given by Garrido et al. (2008) and Prgomet et al. (2019) using a similar solvent extraction method, where it ranged from 9.1-16.1 mg GAE/ 1g dw. and 7.62-25.17 mg GAE/1g dw, respectively. In addition to these, there are also several reported studies in the literature about the determination of polyphenolic content of almond skin (Mandalari et al., 2010; Milbury et al., 2006; Bolling et al., 2010). According to these studies, almond skin has larger quantities of polyphenolics with respect to whole almond. But, there is no consistency among the literature TPC results. A number of factors caused that wide range, including different extraction methods, cultivation, processing, gene, environment, and etc. (Bolling, Chen and Blumberg, 2011).

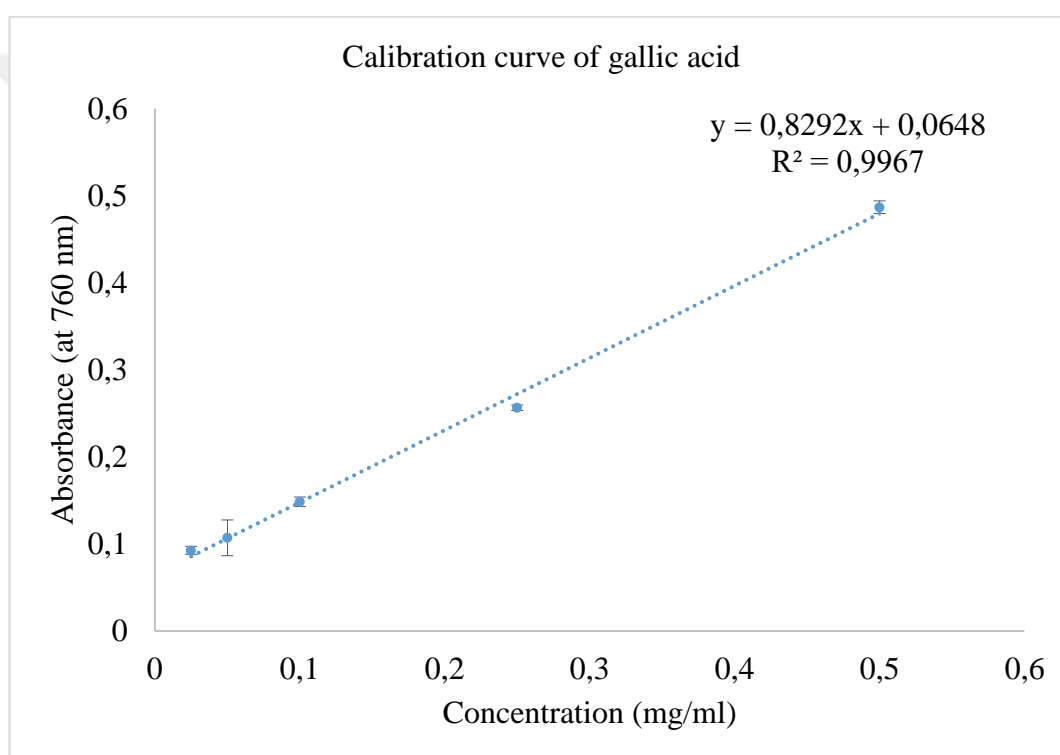


Figure 12. Calibration curve of gallic acid standard. Error bars represent the standard deviations of recorded absorbance values (n=3).

4.2. Film preparation

After the obtaining ASE, neat PLA and PLA-ASE film samples were prepared by using solvent casting method. Prepared film samples as can be seen in Figure 13. Neat PLA, 1.8, 3.2, and 4.6 wt.% PLA-ASE films were nominated as a, b, c, and d, respectively.

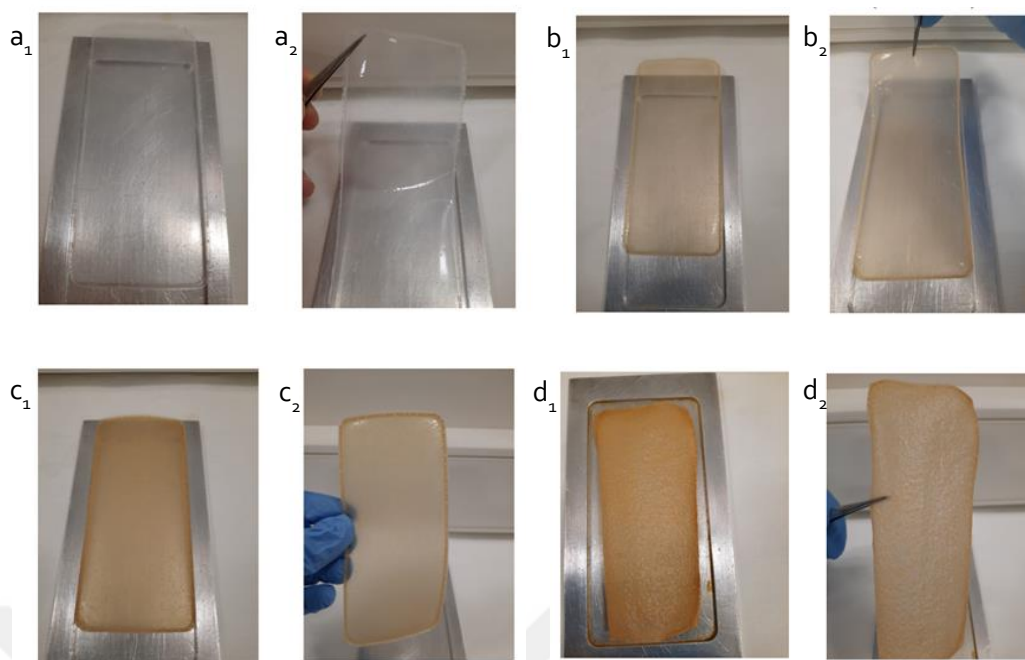


Figure 13. Solvent casted PLA and PLA-ASE film samples. a₁, b₁, c₁, d₁ are films inside the mold. a₂, b₂, c₂, d₂ are self-standing films.

4.3 Characterization of film samples

4.3.1 Optical properties

4.3.1.1 Uv-Vis light barrier properties

Figure 14 shows the transmission of UV-Vis light of the neat PLA and PLA-ASE films as percent transmittance (%). The neat PLA exhibited the highest light transmittance in the range 800-250 nm of the spectrum. On the other hand, PLA-ASE composite film samples showed a significant reduction in the transmittance of light with the incorporation of almond skin extract. Also, values of %T declined depending on the extract amounts in the polymer films. While the neat PLA has a maximum transmittance of around $65 \pm 8.5\%$, the 1.8 wt.% PLA film has a maximum transmittance of around $19 \pm 1.6\%$. With the increase in the amount of ASE, 3.2 wt.% PLA-ASE film and 4.6 wt.% PLA-ASE film overlapped. Their transmittance values were found to be around the highest at $4.5 \pm 1.03\%$. In addition to the extract content, an increase of the film thicknesses in these films also might be an effect on the high descent in transmittance with respect to 1.8 wt.% PLA-ASE. According to these results, it can be assumed that almond skin extract has a noticeable effect on light transmittance. There was a considerable reduction in the UV region (250-400 nm) which comprises UV-A and UV-B lights which cause photochemical degradation

(Auras, Harte and Selke, 2004a). The reduction in transmittance was particularly pronounced at 260 nm, with a decrease from $28 \pm 9.02\%$ (neat PLA) to $0.6 \pm 0.25\%$ (PLA-ASE composites). Considering these, PLA-ASE composite films with the extract could be the better option for light-sensitive products to protect from the photochemical deteriorations caused by UV light. For the food packaging applications, this is a desirable functionality as a UV barrier property (Kanmani and Rhim, 2014). In the literature, there have been many studies of composite films using natural extracts and similar results also have been reported (Manzanarez-López et al., 2011; Hwang et al., 2012; Riaz et al., 2020). The dispersion of the polyphenolic ASE in the PLA matrix could influence the light scattering and caused to reduction in transparency (Figure 15) of PLA-ASE films (Kanmani and Rhim, 2014). Furthermore, increment in the opacity might be a reason for the developed UV-Vis light barrier properties. In addition to these, flavanoids, which are polyphenolics in almond skin, play an important role as UV light absorbers (Prgomet et al., 2017).

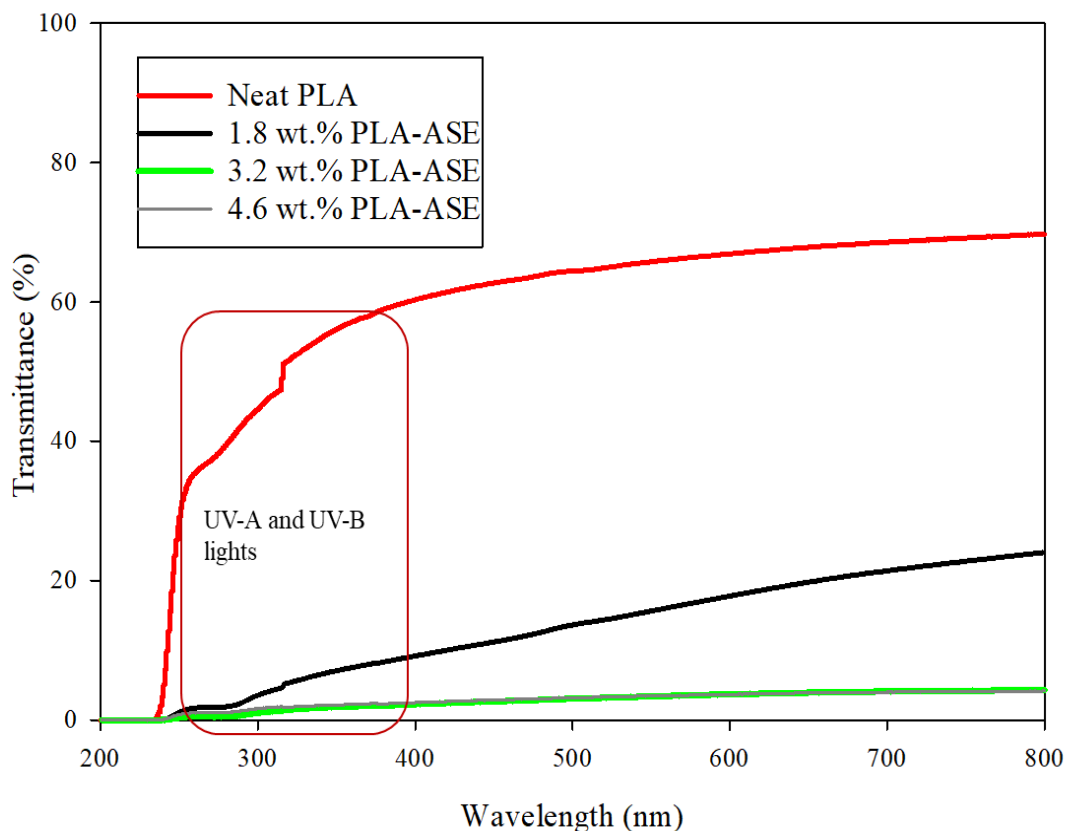


Figure 14. UV-Vis spectra of PLA and PLA-ASE film samples.

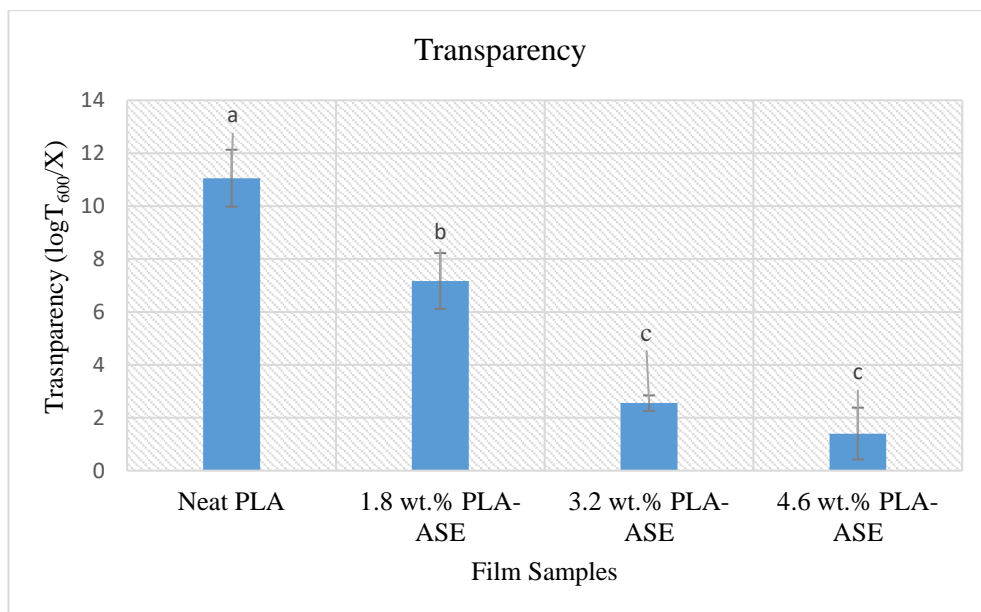


Figure 15. Transparency values of PLA and PLA-ASE film samples. Error bars represent the standard deviations of transparency values (n=3).

The detailed calculations of transparency values and film thicknesses of the film samples were presented in Appendix B.

4.3.1.2 Color

Between the prepared film samples, neat PLA which is the control film showed the highest lightness value (L^*) (85.35) in accordance with the white appearance. While the L^* value decreased to 72.64 with the addition of extract, the a^* and b^* values of neat PLA increased. The yellowish colors seen in the composite films also matched these results. The increment in a^* and b^* values were parallel with the increase in the amount of extract added to the films, however, the value of L^* continued to decrease. All of these were reflected in the total color difference (ΔE^*) of the film samples. With the incorporation of ASE, the total color difference increased to 23.38 in PLA-ASE film samples with respect to neat PLA film. The results of the Hunter Lab color parameters of each film were presented in Table 7. These color changes could be derived from the natural yellowish color of the ASE. Similar results were reported by García et al., (2020) for PCL-based films with the incorporation of almond skin extract. Additionally, it has been suggested that this color change in polymer films which are incorporated natural extract might be through the phenolic pigments in plant-based extracts (Wang et al., 2012). The development of yellowish color in composite films containing natural extracts also was reported with similar results in previous studies

(Wrona et al., 2017; Shankar and Rhim, 2018; Radusin et al., 2019). In addition to these, a lower lightness value (L^*) might be helpful for oxidative protection (Salević et al., 2019).

Table 7. Color test results of the PLA and PLA-ASE film samples.

Parameters	Neat PLA	1.8 wt.% PLA-ASE	3.2 wt.% PLA-ASE	4.6 wt.% PLA-ASE
L^*	85.35	79.28	79.01	72.64
a^*	3.08	4.2	6.26	9.22
b^*	-5.59	10.02	15.66	25.36
ΔE^*	-	13.75	17.26	23.38

4.3.2 Structural analyses (FT-IR)

The FTIR spectra of neat PLA and PLA-ASE composite films were shown in Figure 16. Also, the solution and dried form of ASE were shown in the same figure. The most obvious bands of the PLA IR spectra were sighted in the regions of 3000-2900 cm^{-1} , 2000-1000 cm^{-1} , and 850-650 cm^{-1} . The peaks at 2995 and 2945 cm^{-1} were related to the asymmetric and symmetric stretching vibrations of CH_3 , respectively. In addition to these, the band at 1457 cm^{-1} represented the asymmetric bending vibration of CH_3 . The strong peak at 1749 cm^{-1} represented the stretching vibration of the carbonyl group ($\text{C}=\text{O}$) in PLA molecules. Also, the bending vibrations of this group were shown at 1267 cm^{-1} . The spectra at 1180, 1080, and 1043 cm^{-1} were assigned to the stretching vibration of C-O-C originated from the complex vibrational absorptions due to the ability of connection of C-O with different atoms and functional groups. The bands at 869 and 752 cm^{-1} represent the amorphous and crystalline phases of PLA, respectively (Weng et al., 2013; Darie-Niță et al., 2018).

The most characteristic peaks of almond skin extract were observed at around 3400-3300 cm^{-1} , 3000-2900 cm^{-1} , 1700-1600 cm^{-1} , 1300-1000 cm^{-1} . The peak appearing at 3400-3000 cm^{-1} is related to the O-H stretching vibrations derived from the hydroxyl groups and intermolecular hydrogen bonding of polymeric compounds such as phenols (Hashemian, Salari and Yazdi, 2014). The peak at around 2920-2940 cm^{-1} could be associated with the stretching vibrations of $-\text{CH}_3$ and $-\text{CH}_2$ groups in carboxylic acids which are presented in phenolic acids (Ömeroğlu Ay et al., 2012). The other most

obvious characteristic peaks in the ASE appearing at a range of 1600 -1700 cm^{-1} . In this range, particularly peaks at between 1620-1650 cm^{-1} corresponds to stretching vibrations of C=O and C=C which are typical for the flavonoids. Also, it could be related to the stretching vibrations of C=O because of the aromatic ring deformations (Trifunski et al., 2015; Oliveira et al., 2016). The peaks at around 1000-1300 cm^{-1} are associated with the alcohols, esters, carboxylic acids, and ethers which are found mainly in phenolic compounds (da Silva et al., 2018).

The locations of the PLA characteristic peak did not change in PLA-ASE composite films with the addition of ASE. In addition to these, the decrease in the characteristic peak intensities of PLA was observed in respect of increase of the ASE content. To confirm this, the area under the absorbance peaks was calculated by integral method, and integration of the peak intensities resulted in a decrease from 95.5 to 8.9 with the highest extract amount. The fact that the peaks remain in the same locations with only a decrease in their intensity indicates that no appreciable chemical bond has formed (Wang and Rhim, 2016). This result also might be related to the decrease in the amount of polymer in per unit volume of the film solution. As a result, there was no significant chemical interaction, that would change the chemical structure of the polymer, between PLA solution and ASE. This result was in line with the further findings in thermal properties. Similar results were reported for the LDPE and PLA-based films with grapefruit seed extract (Wang and Rhim, 2016) and PCL-based film with the incorporation of almond skin extract (García et al., 2020).

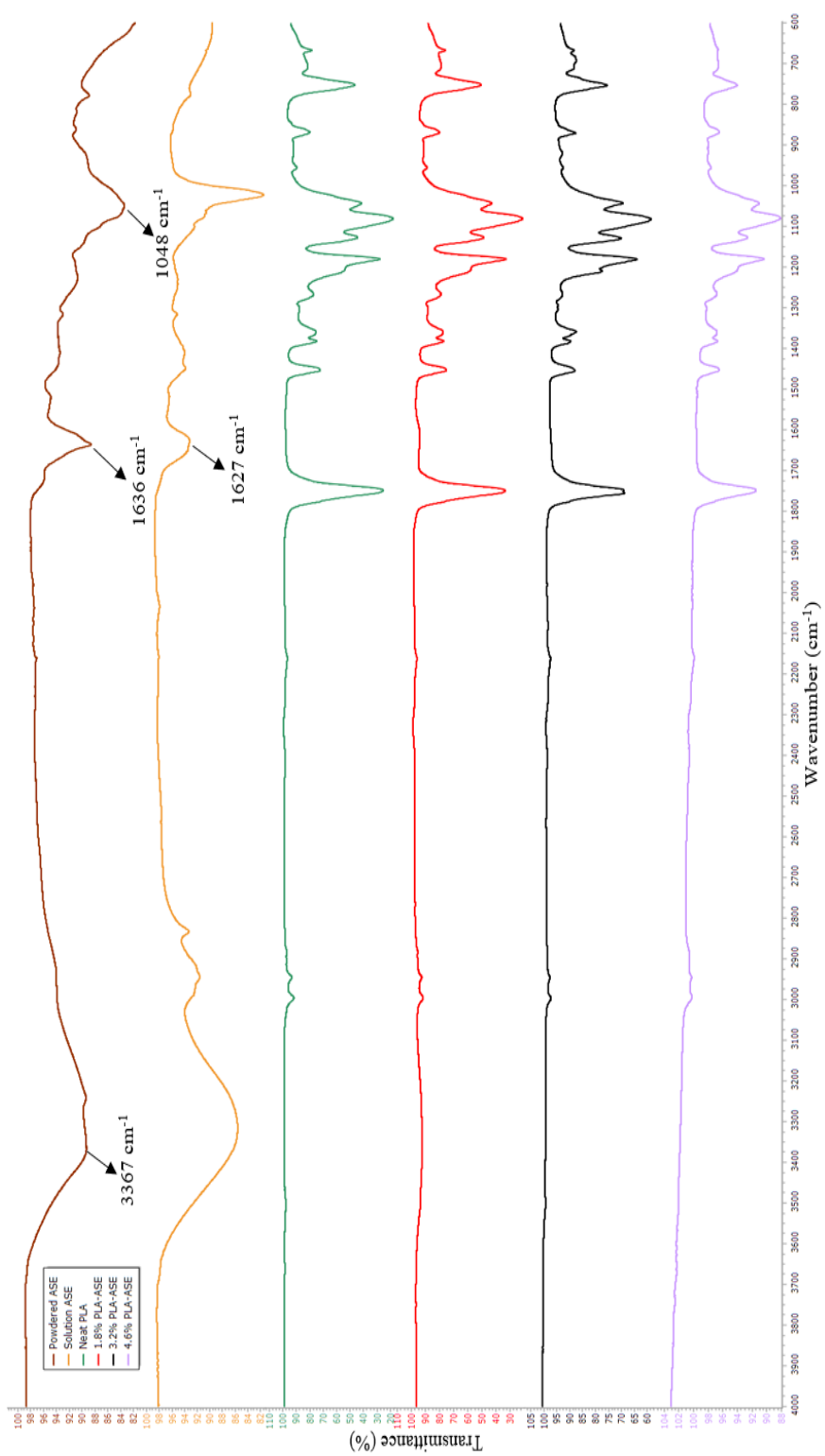


Figure 16. FT-IR spectra of PLA, PLA-ASE film samples, powdered, and solution of ASE. Labels are the characteristic peaks of ASE.

4.3.3 Mechanical properties

Considering the tensile test results of the film samples, mechanical properties were affected by the incorporation of the almond skin extract into PLA polymer film. The results are shown in Table 8 below. In the elastic modulus of PLA-ASE films, a reduction of up to $40 \pm 13\%$ was observed with respect to neat PLA. In the tensile strength values, this decrease reached up to $51 \pm 15\%$. In addition to these, percentages of elongation of the PLA-ASE films decreased, as well. But, a significant decrease which is around $24 \pm 7.3\%$ was seen only in the 4.6 wt.% PLA. The mechanical properties of the polymer films are mainly associated with the crystalline structure and intermolecular interactions in the polymer matrix. The incorporated additives into polymer may interfere with polymer chain arrangement and result in alterations in physical properties such as mechanical (Chambi and Grosso, 2006; Pastor et al., 2013). It is obvious from the SEM analyses of the PLA-ASE films, as the amount of ASE extract added to the polymer matrix increased, unevenness increased and deterioration occurred in the structure with respect to neat PLA film. Therefore, a descent in the mechanical properties of the composite films was observed. In the literature, similar kinds of results were reported by Riaz et al. (2020) involving the development of chitosan-based films with the addition of polyphenolic extract. They suggested that the decrement in mechanical properties related to the deterioration in the crystalline order of the composite film samples. Additionally, humic acid as an antioxidant compound showed similar effects on the modulus of elasticity and tensile strength of the PLA-based films (Bishai et al., 2014). There are several studies about composite films including natural extracts in the literature and the results varied to the polymer matrix and phenolic extracts (Kanmani and Rhim, 2014; Sun et al., 2017; Darie-Niță et al., 2018; García et al., 2020). Due to the lack of studies containing PLA/ASE in the literature, it is difficult to compare the results with different types of materials.

Table 8. Tensile properties (mean \pm SD; n=3) of the neat PLA and PLA-ASE films. Different superscripts in the same row are significantly different ($p < 0.05$).

Property	Neat PLA	1.8 wt.% PLA-ASE	3.2 wt.% PLA-ASE	4.6 wt.% PLA-ASE
Elongation at break (%)	102.93 \pm 28.49 ^a	106.89 \pm 11.03 ^a	91.84 \pm 10.54 ^{ab}	77.27 \pm 13.67 ^b

Table 8 (continued). Tensile properties (mean \pm SD; n=3) of the neat PLA and PLA-ASE films. Different superscripts in the same row are significantly different ($p<0.05$).

Modulus of elasticity (MPa)	606.42 \pm 41.36 ^a	451.35 \pm 80.38 ^b	330.27 \pm 29.47 ^c	305.31 \pm 23.84 ^c
Tensile strength (MPa)	30.05 \pm 3.99 ^a	20.18 \pm 5.45 ^b	13.78 \pm 0.67 ^c	11.61 \pm 2.28 ^c

4.3.4 Gas barrier properties

4.3.4.1 Water vapor permeability (WVP)

Investigation of the barrier properties of the developed film samples is required for desired applications. According to water vapor permeability test results, almond skin extract has led to a significant effect ($p<0.05$) on the WVP of the PLA-ASE film sample (Figure 17). The neat PLA, without additive, showed the highest water vapor barrier property related to the lowest vapor permeability value as $1.49 \times 10^{-7} \pm 3.69 \times 10^{-8}$ gm/m²hPa. The most significant difference in WVP was observed for the 4.6 wt.% PLA film containing the highest ASE content and WVP value was recorded as $2.91 \times 10^{-7} \pm 3.9 \times 10^{-8}$. These permeability changes might be related to the surface structure of the PLA-ASE films. In the SEM images (see in Chapter 4.3.7.1), increment of extract content developed structural discontinuities in the PLA matrix. While the 1.8 wt.% PLA-ASE had a smooth surface similar to neat PLA, it was observed that indented surface structure increased on the 3.2 wt.% and 4.6 wt.% PLA-ASE films. Thus, these results about surface morphology and water vapor barrier property were mutually supportive. Additionally, these changes might be related to the hydrophilic nature of the polyphenols in the almond skin extract content. ASE might have a higher affinity to water than PLA thus it might decrease the water vapor barrier properties of PLA. García et al. (2020) were reported similar results by the addition of the almond skin extract into the PCL matrix. Additionally, in the previous studies, significant differences in water vapor permeability have been reported with the addition of natural extracts to polymer films with similar reasons (Pastor et al., 2013; Kanmani and Rhim, 2014; Salević et al., 2019).

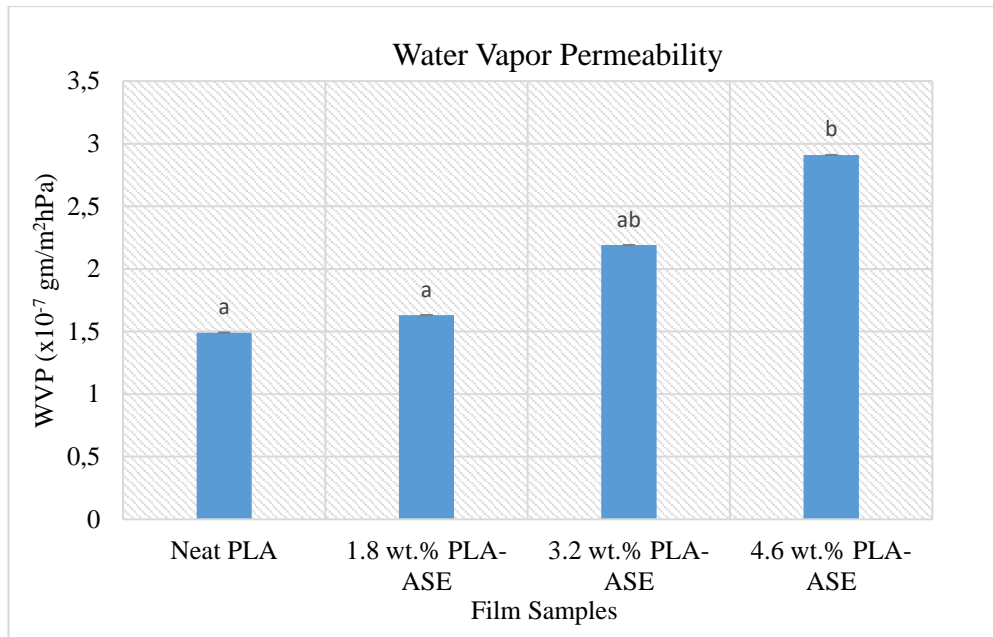


Figure 17. WVP values of neat PLA and PLA-ASE film samples. The different superscripts are significantly different ($p < 0.05$). Error bars represent the standard deviations ($n=3$).

The detailed calculations of WVP values were presented in Appendix C.

4.3.4.2 Oxygen permeability

Determination of peroxide value was used to evaluate the effect of the antioxidant extract on the oxygen permeability of the film samples. This value gives the number of hydroperoxides which are primary lipid oxidation products in a tested sample (Yildiz, Wehling and Cuppett, 2003). In this study, the highest peroxide value was determined for the neat PLA film as 58.27 ± 1.5 meq O_2 /kg. For the composite PLA-ASE film samples, lower peroxide values were measured (Figure 18). Peroxide values of the PLA-ASE film samples are 29.86 ± 1.5 , 37.86 ± 8.7 , 35.33 ± 7.5 meq O_2 /kg for the 1.8 wt.%, 3.2 wt.%, 4.6 wt.% PLA film samples, respectively. Even the lowest extract amount provided a decrease of almost $48.5 \pm 2.8\%$ percent in peroxide value, the increasing amount of extract has no significant effect on the oxygen barrier property as peroxide value. Similar results were reported by Martins et al. (2018). They researched the effect of green tea extract as a natural antioxidant on the PLA film and they examined that the lowest concentration of the extract showed the maximum effect. They suggested as a reason that higher extraction content may show a prooxidant effect. In addition to this, Samsudin, Soto-Valdez and Auras, (2014) were reported that light also had an accelerating effect on oxidation. And because of the

high transparency of neat PLA film, the oil sample in the pure PLA had more PV. They used plant-based (marigold flower) antioxidant extract and incorporated it into PLA matrix and recorded a decrease of around 20% of PV in PLA composite film including the extract.

Concerning the acceptable PV value as around 10-20 meq/kg for vegetable oils (Gordon, 2004), PLA-ASE films could be a promising packaging material option providing longer shelf life by decreasing the oxidation reaction with respect to neat PLA film.

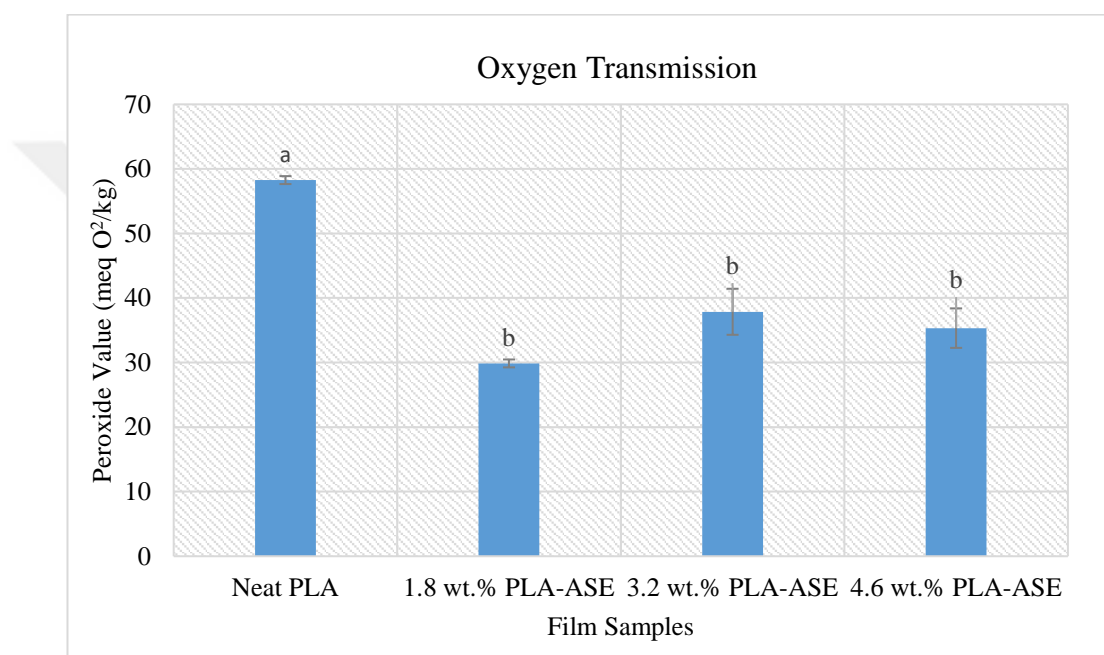


Figure 18. Results of the peroxide value assay for the oil samples covered with PLA and PLA-ASE films. Different superscripts are significantly different ($p < 0.05$). Error bars represent the standard deviations ($n=3$).

The detailed calculations of PV for the oxygen permeability were presented in Appendix D.

4.3.5 Radical scavenging activity (RSA)

DPPH radical scavenging analysis was used to investigate the possible antioxidant activity of PLA-ASE films. The test principle is based on the quenching of free radicals in the presence of antioxidants, lightening the color of the solution and resulting in a decrease in the reading absorbance values (Enayat and Banerjee, 2009). According to the test results, neat PLA film showed almost no antioxidant activity ($0.7 \pm 0.4\%$). In addition to this, the antioxidant activity of the films, as RSA, enhanced with increasing

of the extract concentration as shown in Figure 19. The most significant rise was observed in 4.6 wt.% PLA-ASE film as $22 \pm 4.8\%$ RSA. Other concentrations (1.8 wt.% and 3.2 wt.%) may have had a less RSA effect due to their lower content of ASE. Additionally, DPPH analysis is just one of the antioxidant activity determination methods. To give a precise and general suggestion, only DPPH may not be sufficient. For further studies, it may be necessary to use other antioxidant activity analysis methods such as oxygen radical absorbance capacity assay (ORAC), total peroxyl radical trapping antioxidant parameter (TRAP), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method and etc. (Bedlovičová et al., 2020).

Overall, concerning the DPPH results, the addition of ASE to the PLA matrix provided radical scavenging activity due to the polyphenolic compounds that are found in almond skin (Valdés et al., 2015). Similar results were examined for a chitosan-based film with green tea extract (Siripatrawan and Harte, 2010), alginate films incorporated with ginseng extract (Norajit, Kim and Ryu, 2010), antioxidant PLA composite with lignin (Domínguez-Robles et al., 2019), and PCL film with almond skin extract (García et al., 2020). Interactions between the polymer matrix (PLA) and additive compounds (ASE) play a major role in the alteration of the functional properties of the film. These interactions were also mentioned in the section of FT-IR analyses.

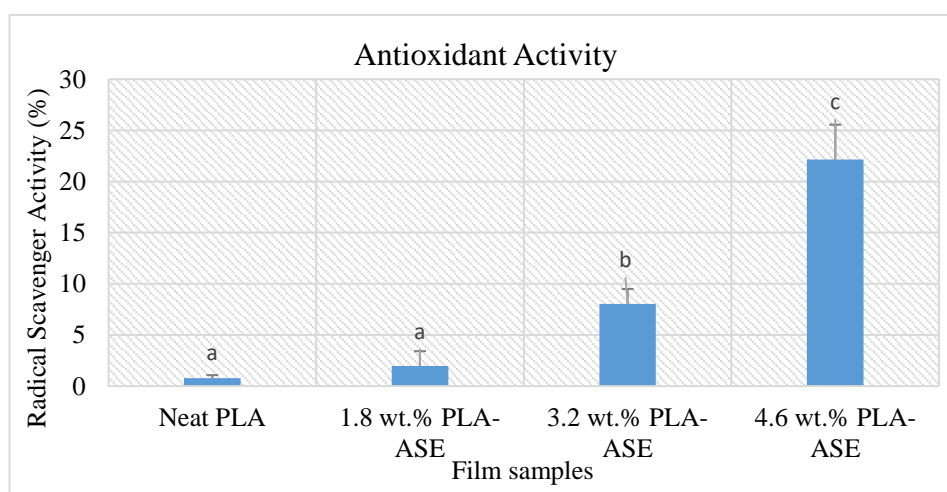


Figure 19. Antioxidant activity values of neat PLA and PLA-ASE film samples. The different letters on the bars are significantly different ($p < 0.05$). Error bars represent the standard deviations ($n=2$).

The detailed calculations of RSA values were presented in Appendix E.

4.3.6 Thermal properties

To investigate the effect of ASE addition on the PLA-ASE films, DSC analyses were carried out. Neat PLA and other composite films including ASE presented almost the same characteristic curves. There were just slight shifts in the values of thermal transition points on the DSC thermograms (Figure 20, 21, 22, 23). This means that the addition of extract could increase the mobility of polymer chains and enables more recombination (Piorkowska et al., 2006). In addition to these, incorporation of ASE did not significantly affect the thermal transition points of PLA ($p > 0,05$) (Table 9). This might be originated from small amounts of the extract added. These results also could be attributed to recrystallization of ASE on PLA surface and therefore partial separation from the polymer matrix as reported before by Jamshidian, Tehrany, et al. (2012). Similar results were reported for the incorporation of ASE to the PCL matrix (García et al., 2020). Overall, no significant change in thermal properties might be advantageous in terms of production and processing.

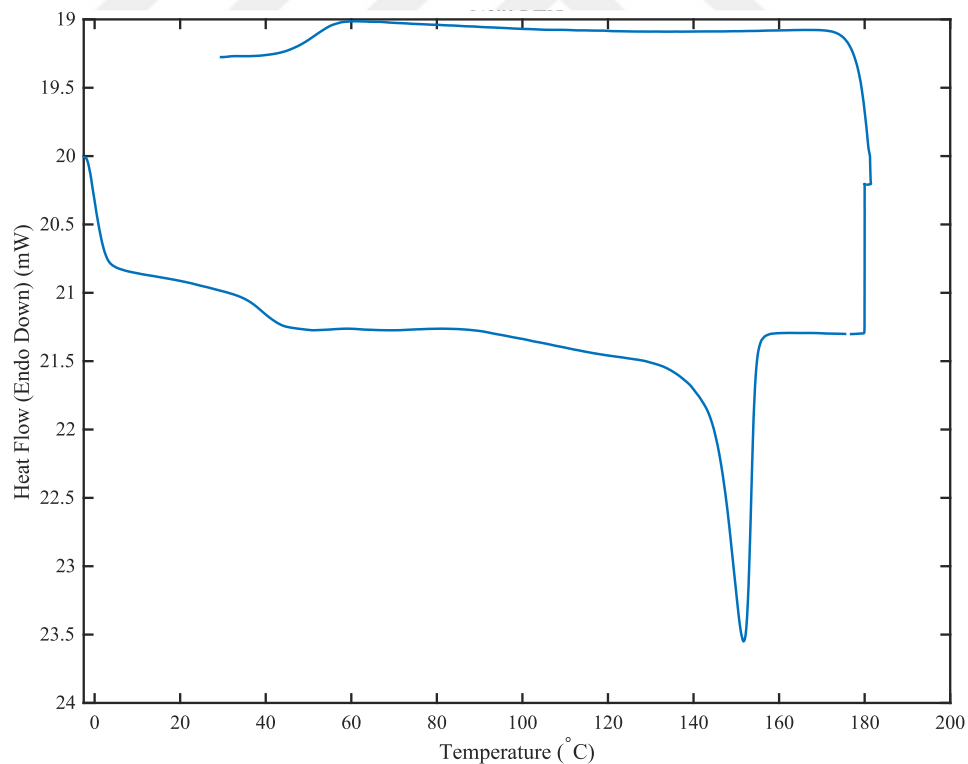


Figure 20. DSC thermogram of neat PLA film sample.

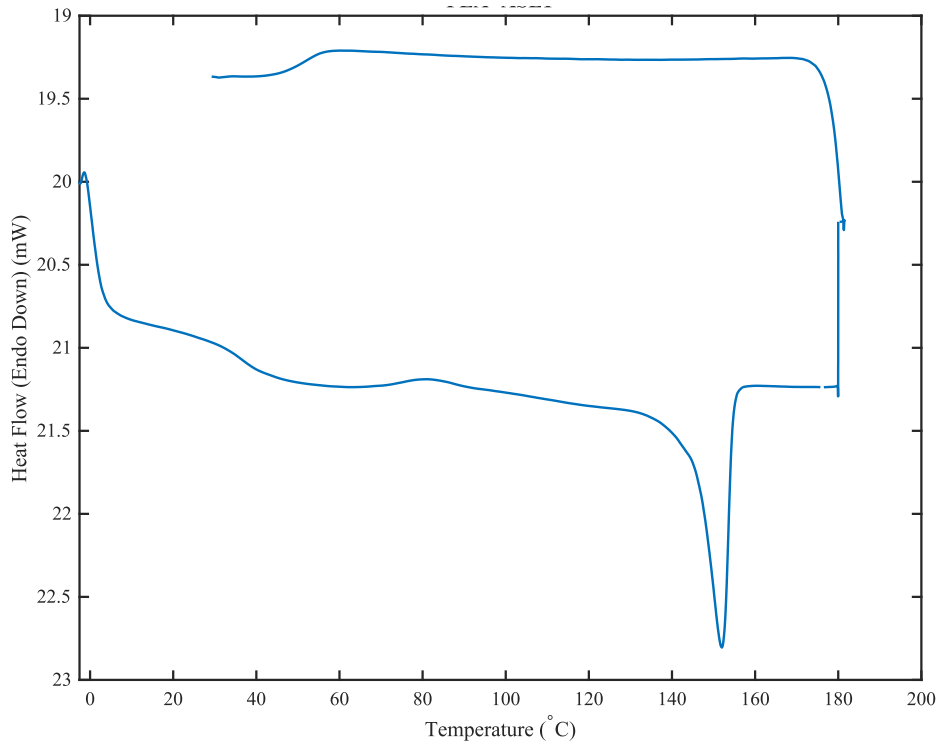


Figure 21. DSC thermogram of 1.8 wt.% PLA-ASE film sample.

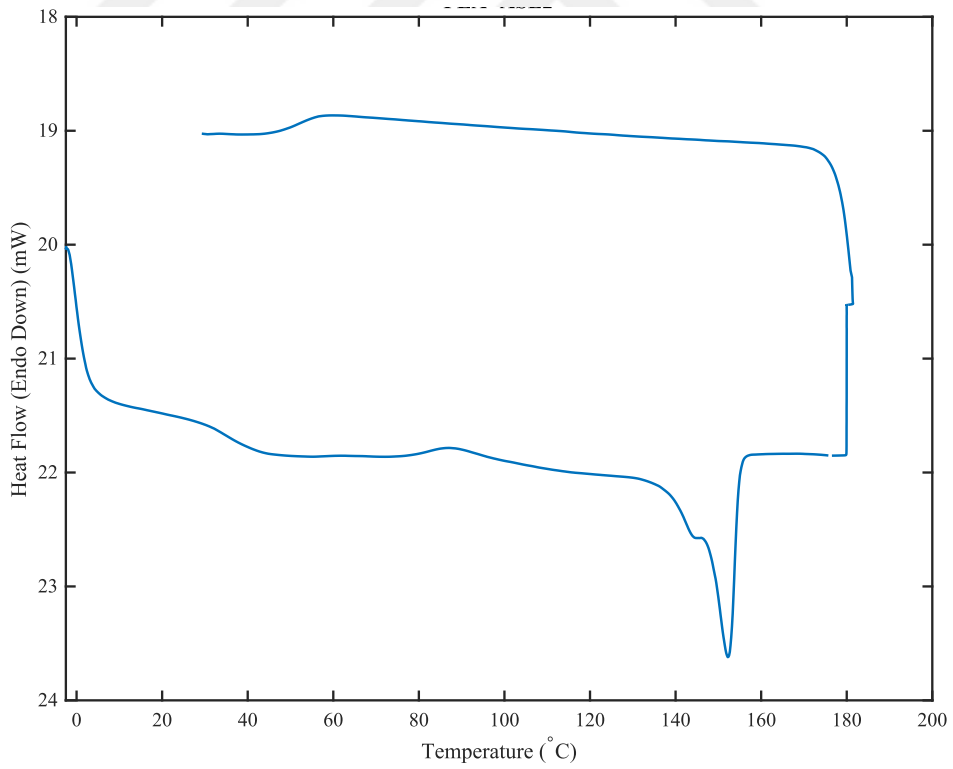


Figure 22. DSC thermogram of 3.2 wt.% PLA-ASE film sample.

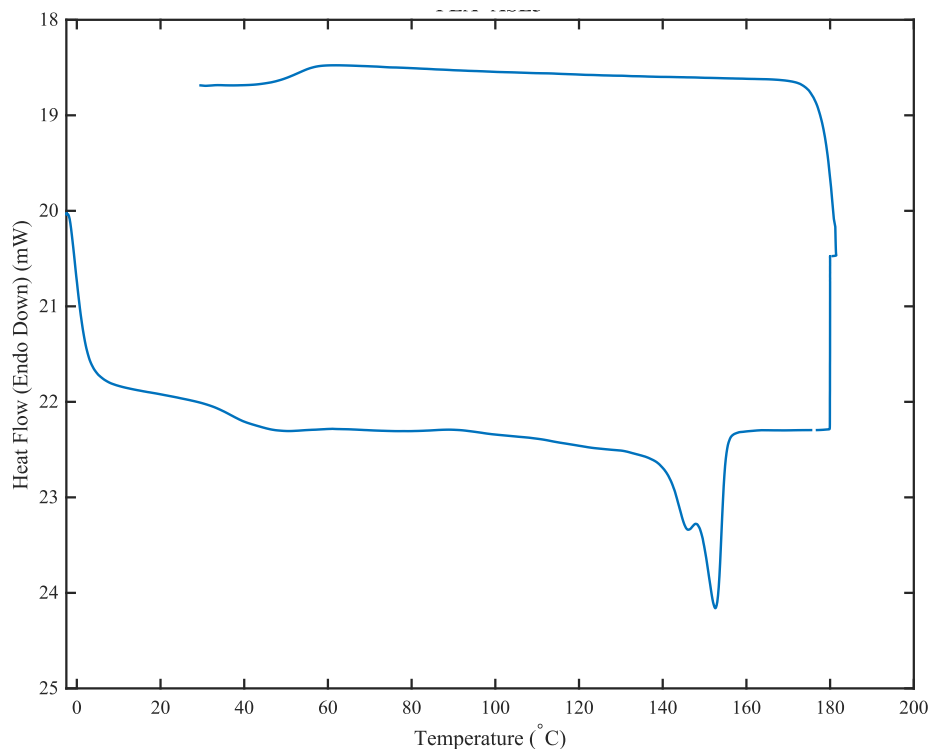


Figure 23. DSC thermogram of 4.6 wt.% PLA-ASE film sample.

Table 9. Thermal transition points (mean \pm SD; n=2) obtained from DSC of PLA-ASE films. Different superscripts in the same row are significantly different ($p < 0.05$).

Thermal Property	Neat PLA	1.8 wt.% PLA-ASE	3.2 wt.% PLA-ASE	4.6 wt.% PLA-ASE
Glass Transition Temperature (T _g), (°C)	41.55 \pm 4.01 ^a	35.54 \pm 0.93 ^a	34.29 \pm 0.13 ^a	38.23 \pm 2.34 ^a
Melting Temperature (T _m), (°C)	151.66 \pm 0.70 ^a	152.25 \pm 0.34 ^a	152.17 \pm 0.34 ^a	152.45 \pm 0.14 ^a
Crystallinity (X _c ,%)	38.23 \pm 2.42 ^a	36.02 \pm 2.58 ^a	38.82 \pm 1.61 ^a	39.93 \pm 1.14 ^a

The detailed calculations of X_C values were presented in Appendix F.

4.3.7 Morphological analyses

4.3.7.1 SEM

SEM images of the surface of the neat PLA film and composite films containing almond skin extract are shown in Figure 24. According to SEM images, neat PLA was

considerably smooth and homogenous. The 1.8 wt.% PLA-ASE film, containing the lowest amount of antioxidant extract, exhibited a quite similar structure to the neat PLA film. There was only minor unevenness on its surface. As the amount of extract in the composite films increased, an indented and more heterogeneous structure was formed as shown in Figure 24 (c, g, and d, h). These discontinuities that occurred in the homogeneous structure of the polymer matrix might be caused by the hydrophilicity of polyphenolic compounds present in the almond skin extract. In the image of 4.6 wt.% PLA-ASE film (Figure 24, d, h), the formation of the possible small voids about $16.5 \pm 2.1 \mu\text{m}$ in diameter indicated poor bonding or debonding between polymer matrix and extract due to the excessive amount of extract. Similar results were reported by Giita Silverajah et al. (2012), Bishai et al. (2014), García et al. (2020) and Riaz et al. (2020). For functional packaging applications, the porous structures may contribute to the possible release of antioxidants. In this regard, more precise information about the presence of porous could be obtained by taking cross-sectional images in further studies. In addition to that, void formation and porous structure cause a descent in gas barrier properties (Wu et al., 2014). Considering these, significant decrease in the water vapor barrier of the 4.6 wt.% PLA-ASE film sample (Chapter 4.2.4.1) also might be related to this result.

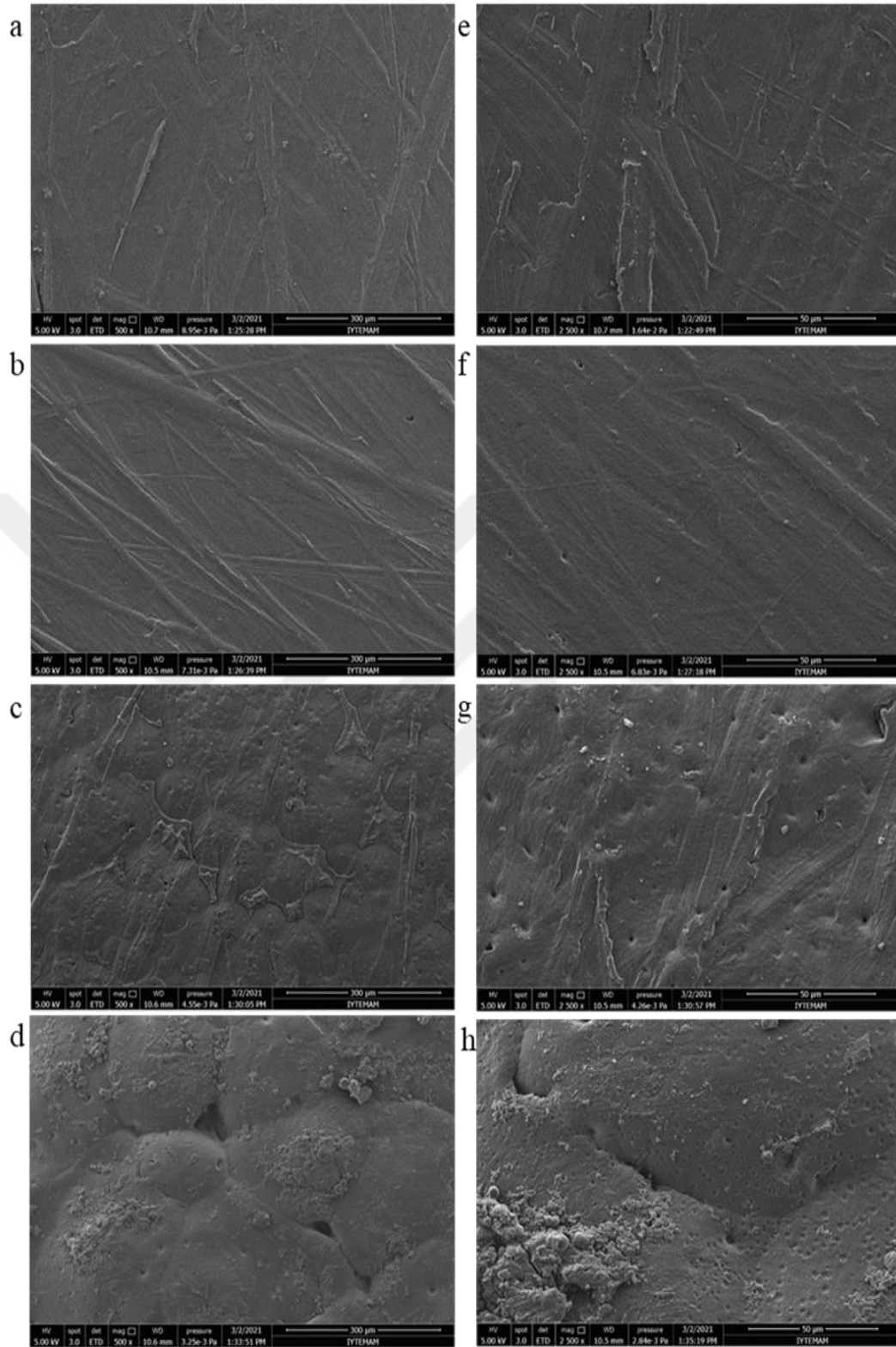


Figure 24. SEM surface micrographs of PLA and PLA-ASE films. (a,e) Neat PLA; (b,f) 1.8 wt.% PLA-ASE; (c,g) 3.2 wt.% PLA-ASE; (d,h) 4.6 wt.% PLA-ASE. The left column's magnification: 500x; scale bar: 300 μm , the right column's magnification: 2500x; scale bar: 50 μm .

4.3.7.2 AFM

To investigate the surface morphology of the film samples, AFM tapping mode was performed. Root means square (RMS) values, which are the roughness indices, were measured (Figure 25). Topographical images ($5 \times 5 \mu\text{m}^2$) of the neat PLA and PLA-ASE film samples are shown in Figure 26. Also, topographical images ($10 \times 10 \mu\text{m}^2$) of the neat PLA and PLA-ASE film samples are shown in Figure 27. According to the results, the addition of antioxidant extract to PLA film affected the surface microstructure. The Neat PLA film had a relatively smooth surface ($\text{RMS} = 1.98 \times 10^1 \pm 0.6 \text{ nm}$) compared to composite PLA films. However the 1.8 wt.% PLA-ASE film also presented similar homogenous surface property, its roughness increased ($\text{RMS} = 6.21 \times 10^1 \pm 5.09 \text{ nm}$). The AFM RMS values and SEM images complemented each other. The roughness value of 3.2 wt.% PLA-ASE film ($\text{RMS} = 9.45 \times 10^1 \pm 2.1 \text{ nm}$) increased with the increasing amount of extract. As it is shown in the image, it presented a quite rough and heterogeneous surface structure. Unfortunately, for the 4.6 wt.% PLA-ASE film sample, we could not get reliable results due to the tip problems. The reason for that might have been the tip could not approach adequately because of the indented and heterogeneous surface of the film.

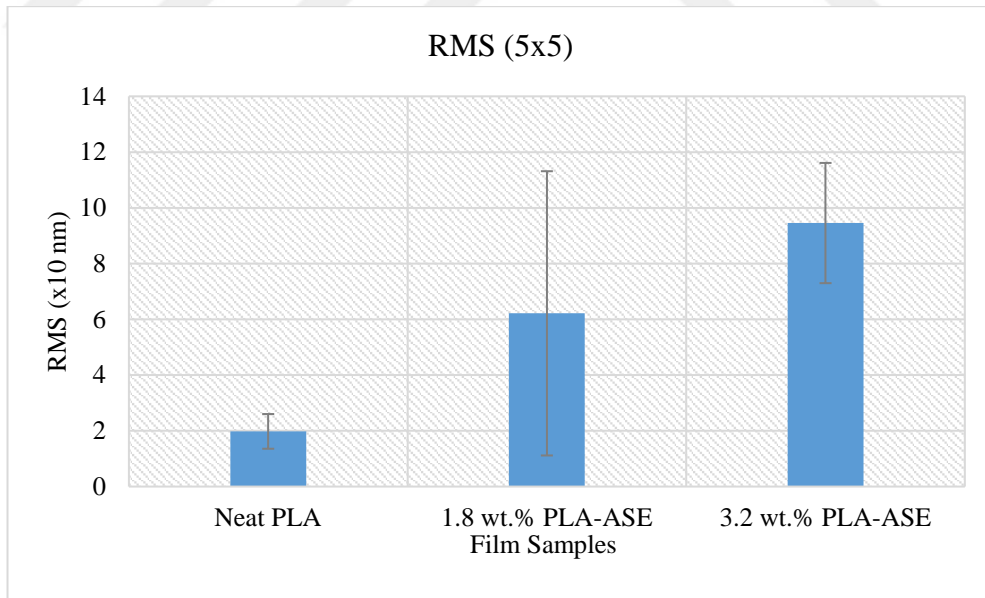


Figure 25. RMS values of PLA and PLA-ASE film samples for $5 \times 5 \mu\text{m}^2$. Error bars represent the standard deviations ($n=2$).

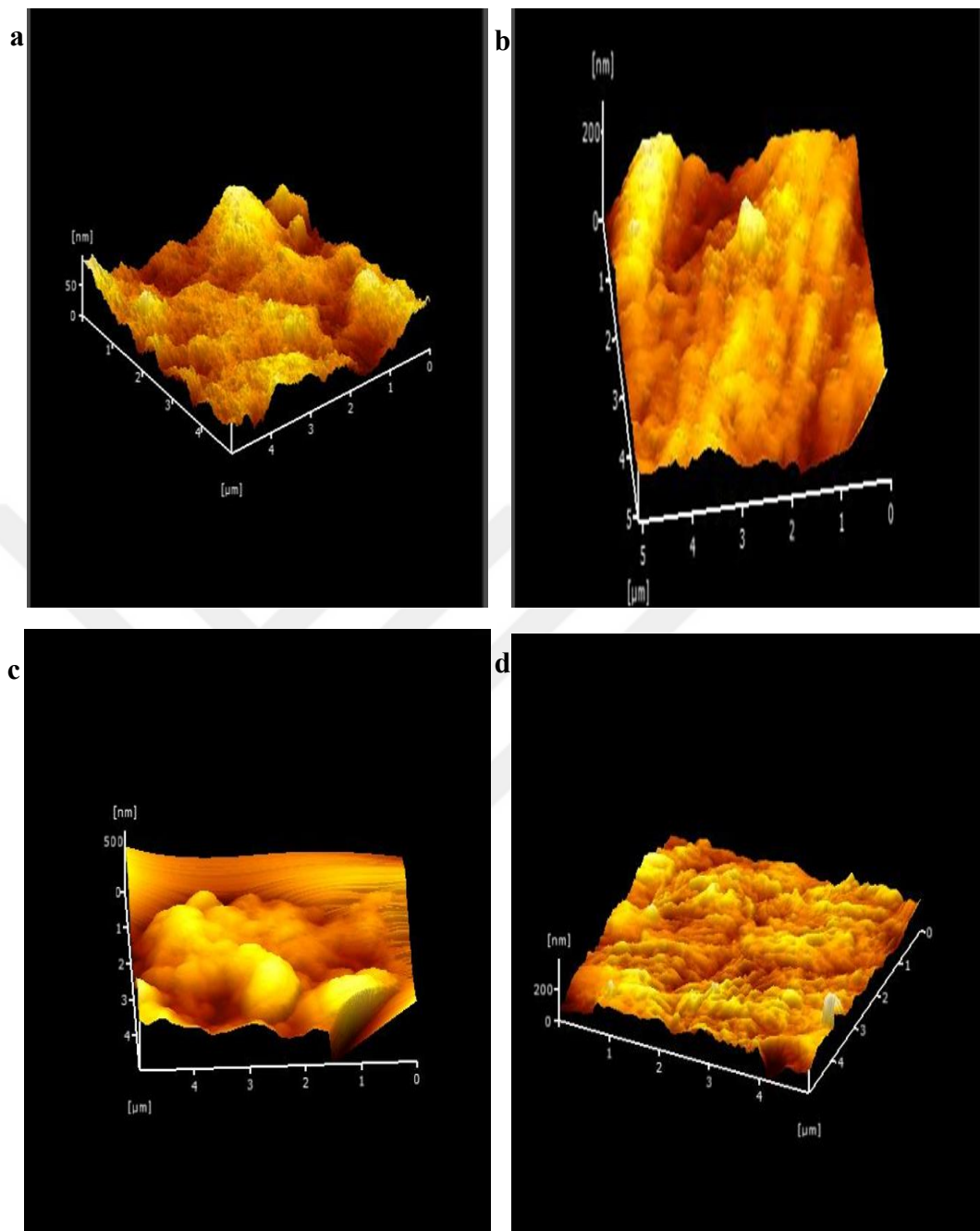


Figure 26. 3D AFM images of PLA and PLA-ASE films for area $5 \times 5 \mu\text{m}^2$. Neat PLA, 1.8, 3.2, and 4.6 wt.% PLA-ASE films are a, b, c, d, respectively.

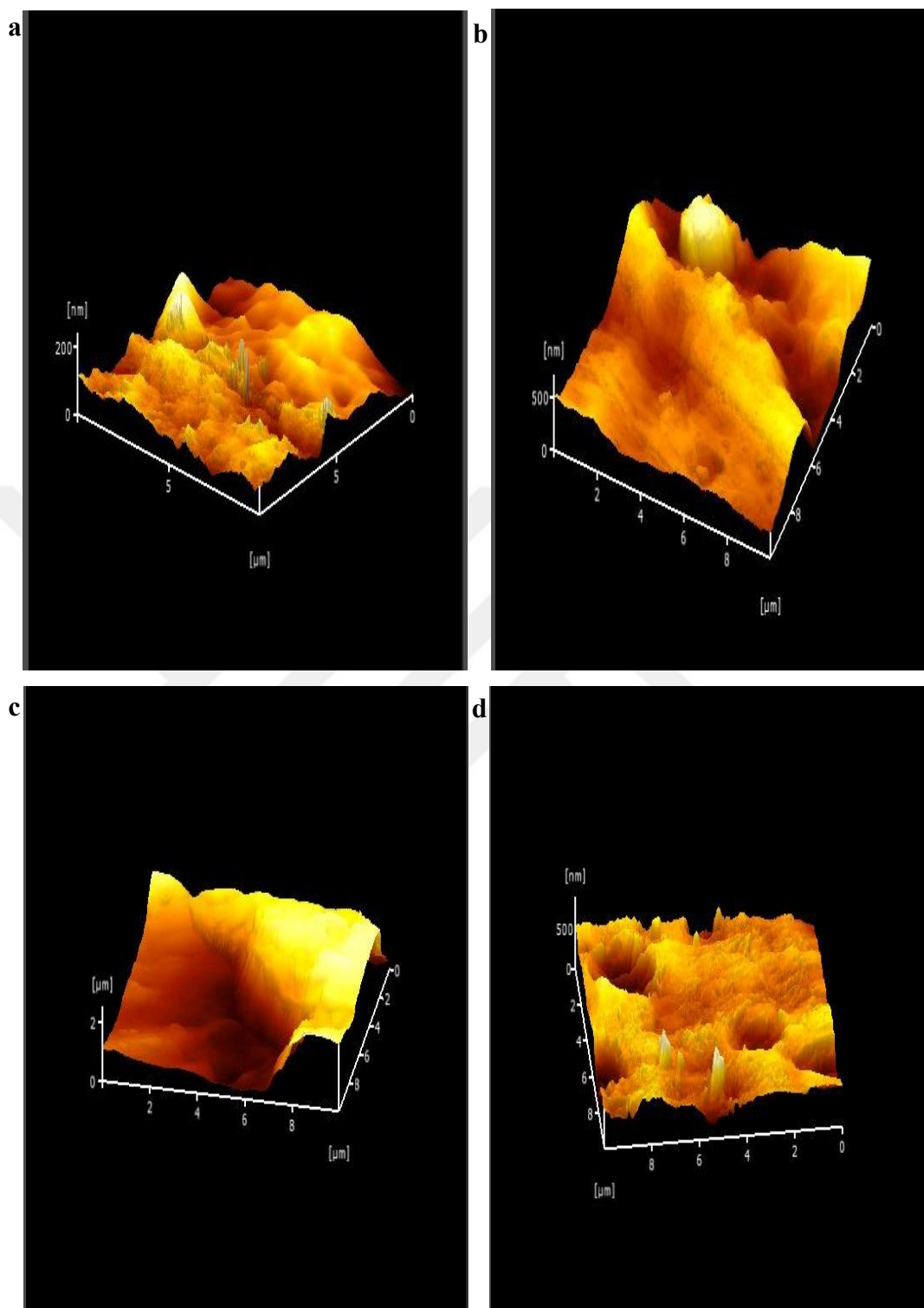


Figure 27. 3D AFM images of PLA and PLA-ASE films for area $10 \times 10 \mu\text{m}^2$. Neat PLA, 1.8, 3.2, and 4.6 wt.% PLA-ASE films are a, b, c, d, respectively.

CHAPTER 5: CONCLUSION

The major objective of this thesis was to develop a functional composite polymer film with the addition of natural extract containing polyphenolic compounds. For this purpose, firstly the natural extract was obtained from the almond skin. Then, the total polyphenolic compound content of ASE was determined. Then, to develop the PLA-ASE film samples, the solvent casting method was used. Finally, morphological, structural, thermal, mechanical, barrier and antioxidant activity analyses of the developed films were performed to investigate the functional properties of PLA-ASE films.

For the natural extract, almond skin was chosen for the recovery of its valuable polyphenolic compounds. As a fruit, almond has widespread production and consumption both in our country and in the world. Because of the valuable content of the whole part of the almond, its recovery is a notable issue. For the extraction of bioactive polyphenolic compounds from the almond skin, a solvent extraction method was used. The result of the determination of total polyphenolic content was reported as 6.53 ± 0.07 mg GAE/1g dw which means 6.53 mg gallic acid equivalent per gram of the dry skin with a yield of 18.75%. This result was consistent with the data in the literature and showed that this almond skin extraction was successful. Moreover, the characteristic chemical peaks belonged to almond skin and polyphenolic compounds were observed in the FTI-IR analyses of the extraction powder and solution.

The natural ASE obtained by the extraction step was added to the PLA at three different concentrations as 1.8, 3.2, and 4.6 wt.%. Thus, three different PLA-ASE composite film samples and the neat PLA (without ASE) were prepared. To understand the effect of ASE on PLA, further characterization steps were performed.

For the surface morphology analyses of the PLA-ASE film samples, SEM and AFM images were taken. According to SEM images, an increase in extract amount caused more distinct porosity and cavities on the film surface. This porous structure might be an advantage for the functional packaging systems for the release of the active compounds. However, the void formation on the surface could lead to descent of some barrier properties. This situation was observed more specifically for the 4.6 wt.% PLA-ASE film in accordance with the increase in water vapor permeability. In addition to these, to investigate the roughness of the film surfaces root mean square (RMS) values

were measured with the AFM and also topographic images were taken. In the AFM images, a more indented and heterogeneous surface structure was observed similar to SEM images. Additionally, RMS values of PLA-ASE films increased with the addition of ASE but there was no reliable result for the 4.6 wt.% PLA-ASE. This situation was caused by some tip problems. Due to the financial deficiency, an appropriate tip could not be purchased. It has been stated as a point to be considered in future studies that the tip used in this study is not suitable for PLA-ASE film containing greater than the concentration of 3.2 wt.% PLA.

To investigate the structural differences such as chemical interactions between the PLA and the ASE, FT-IR analyses were done. According to FT-IR graphs, there was no difference considerably between the neat PLA film and the PLA-ASE composite films. Characteristic peaks of the PLA remained at the same locations. This was might be due to the addition of the low amount of the ASE.

To understand the functional properties of the composite PLA-ASE films, a variety of experiments were carried out such as mechanical, gas, and light barrier, thermal, morphological, structural, and antioxidant activity. The addition of natural antioxidant extract caused descent in the mechanical test results of the composite films in regard to neat PLA film without the extract. Considering the SEM images, the increment of the extract addition caused unevenness and void formation on the polymer matrix and the crystalline structure has been destroyed. These results have not a negative effect alongside the other functionalities.

Optical properties of the film are one of the important criteria for the packaging film application. To investigate these, UV-Vis screening and transparency calculations were made for the neat PLA and PLA-ASE composite films. Also, PLA-ASE film samples showed lower light transmission in the UV-Vis spectrum. As a packaging material, this was a favorable achievement for light-sensitive products. Additionally, the transparency of the film samples decreased with the addition of the ASE. Also, this might have been a factor in light protectiveness because of its UV absorber phenolic pigments. In addition to these, when the natural colorants in the ASE were considered, the color change of the film was quite possible. Total color changes (ΔE^*) of the films are proportional with the increasement of the extract amount. The yellowish color was observed in the composite films.

The effect of the ASE on the gas barrier properties of the PLA films was examined for water vapor permeability and oxygen permeability. The water vapor barrier property of PLA-ASE films significantly decreased with the highest ASE concentration. The reason for that might be the more cavities and void formation in the 4.6 wt.% PLA-ASE film. This pointed that increase in ASE might lead to a decrease in the WVP. Besides, the oxygen barrier property of PLA-ASE films improved independently from ASE concentration. This result, which would reduce oxidative degradation, shows the possibility of having antioxidant properties.

To measure the antioxidant activity of the film samples, DPPH radical scavenging test method was used. In this test, which is based on the reduction of free radicals, while the pure PLA had no antioxidant activity, the considerable difference was seen mostly in 4.6 wt.% PLA-ASE film containing the highest extract content. Overall, ASE provided radical scavenging activity to PLA films due to its polyphenolic content.

Considering the differential scanning calorimetry test results, the addition of ASE did not change significantly the thermal transition points of PLA-ASE films with respect to neat PLA. This might be a benefit for processing and applications.

Consequently, the results show that this work achieved the aim of the develop functional composite polymer film. ASE was effectively incorporated into PLA and prepared PLA-ASE films have been shown as promising functional packaging materials to minimize oxidative deterioration and maintain the quality of food products for a longer time. Furthermore, it has been a challenging approach to reevaluate almond skin as a by-product contributing to the sustainable environment.

This study might be the first fundamental step for further studies to scale up for industrial production. Also, further studies might be in need of more detailed analyses of antioxidant activity and focus on the application of the PLA-ASE films to food products. Furthermore, migration studies and antimicrobial activity analyses might be performed.

REFERENCES

Adsul, M. G., Varma, A. J. and Gokhale, D. V. (2007) *Lactic acid production from waste sugarcane bagasse derived cellulose*, Green Chemistry, 9(1), pp. 58–62. doi: 10.1039/b605839f.

Agrawal, C. M., Pennick, A., Wang, X. and Schenck, R. C. (1997) *Porous-coated titanium implant impregnated with a biodegradable protein delivery system*, Journal of Biomedical Materials Research, 36(4). doi:10.1002/(SICI)1097-4636(19970915)36:4<516::AID-JBM9>3.0.CO;2-H.

Ahmed, J. and Varshney, S. K. (2011) *Polylactides-chemistry, properties and green packaging technology: A review*, International Journal of Food Properties, 14(1), pp. 37–58. doi: 10.1080/10942910903125284.

Ambrosio-Martín, J., Fabra, M. J., Lopez-Rubio, A. and Lagaron, J. M. (2015) *Melt polycondensation to improve the dispersion of bacterial cellulose into polylactide via melt compounding: enhanced barrier and mechanical properties*, Cellulose. Kluwer Academic Publishers, 22(2), pp. 1201–1226. doi: 10.1007/s10570-014-0523-9.

Anderson, K. S., Schreck, K. M. and Hillmyer, M. A. (2008) *Polymer Reviews Toughening Polylactide Toughening Polylactide*, Polymer Reviews, 48(1), pp. 85–108. doi: 10.1080/15583720701834216.

Armentano, I., Bitinis, N., Fortunati, E., Mattioli, S., Rescignano, N., Verdejo, R., Lopez-Manchado, M. A. and Kenny, J. M. (2013) *Multifunctional nanostructured PLA materials for packaging and tissue engineering*, Progress in Polymer Science. Elsevier Ltd, 38(10–11), pp. 1720–1747. doi: 10.1016/j.progpolymsci.2013.05.010.

Arrieta, M. P., Fortunati, E., Dominici, F., Rayón, E., López, J. and Kenny, J. M. (2014) *PLA-PHB/cellulose based films: Mechanical, barrier and disintegration properties*, Polymer Degradation and Stability, 107, pp. 139–149. doi: 10.1016/j.polymdegradstab.2014.05.010.

ASTM-D882 (1997) in *Standard test method for tensile properties of thin plastic sheeting*. In Annual Book of ASTM Standards; American Society for Testing and Materials. Philadelphia, PA, USA,.

Athanasidou, K. (1996) *Sterilization, toxicity, biocompatibility and clinical*

applications of polylactic acid/ polyglycolic acid copolymers, *Biomaterials*, 17(2). doi: 10.1016/0142-9612(96)85754-1.

Auras, R. A., Harte, B., Selke, S. and Hernandez, R. (2003) *Mechanical, physical, and barrier properties of poly(lactide) films*, *Journal of Plastic Film and Sheeting*, 19(2), pp. 123–135. doi: 10.1177/8756087903039702.

Auras, R., Harte, B. and Selke, S. (2004a) *An overview of polylactides as packaging materials*, *Macromolecular Bioscience*, 4(9), pp. 835–864. doi: 10.1002/mabi.200400043.

Auras, R., Harte, B. and Selke, S. (2004b) *Effect of water on the oxygen barrier properties of poly(ethylene terephthalate) and polylactide films*, *Journal of Applied Polymer Science*, 92(3), pp. 1790–1803. doi: 10.1002/app.20148.

Auras, R., Singh, P. and Singh, J. (2005) *Evaluation of OPLA polymers with existing PET and OPS for fresh food service containers*, *Global Plastics Environmental Conference 2005: GPEC 2005 - Creating Sustainability for the Environment*, (May), pp. 141–156.

Bedlovičová, Z., Strapáč, I., Baláž, M. and Salayová, A. (2020) *A brief overview on antioxidant activity determination of silver nanoparticles*, *Molecules*. MDPI AG. doi: 10.3390/molecules25143191.

Bertolino, M., Belviso, S., Dal Bello, B., Ghirardello, D., Giordano, M., Rolle, L., Gerbi, V. and Zeppa, G. (2015) *Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt*, *LWT - Food Science and Technology*. Academic Press, 63(2), pp. 1145–1154. doi: 10.1016/j.lwt.2015.03.113.

Bishai, M., De, S., Adhikari, B. and Banerjee, R. (2014) *A comprehensive study on enhanced characteristics of modified polylactic acid based versatile biopolymer*, *European Polymer Journal*. Elsevier Ltd, 54(1), pp. 52–61. doi: 10.1016/j.eurpolymj.2014.01.027.

Bismarck, A., Aranberri-Askargorta, I., Springer, J., Lampke, T., Wielage, B., Stamboulis, A., Shenderovich, I. and Limbach, H. H. (2002) *Surface characterization of flax, hemp and cellulose fibers; Surface properties and the water uptake behavior*, *Polymer Composites*, 23(5), pp. 872–894. doi: 10.1002/pc.10485.

Bocchini, S. and Camino, G. (2012) *Flammability and Thermal Stability in Clay/Polyesters Nano-Biocomposites*, Green Energy and Technology. Springer, London, 50, pp. 265–285. doi: 10.1007/978-1-4471-4108-2_10.

Bolling, B. W., Chen, C.-Y. O. and Blumberg, D. L. M. and J. B. (2011) *Tree nut phytochemicals: composition, antioxidant capacity, bioactivity, impact factors. A systematic review of almonds, Brazils, cashews, hazelnuts, macadamias, pecans, pine nuts, pistachios and walnuts*, Nutrition Research Reviews, 24, pp. 244–275. Available at: https://www.cambridge.org/core/services/aop-cambridge-core/content/view/F28DC9CF2246C09ACEFBDF41623CAB63/S095442241100014Xa.pdf/tree_nut_phytochemicals_composition_antioxidant_capacity_bioactivity_impact_factors_a_systematic_review_of_almonds_brazils_cash (Accessed: 15 January 2021).

Bolling, B. W., Dolnikowski, G., Blumberg, J. B. and Chen, C. Y. O. (2010) *Polyphenol content and antioxidant activity of California almonds depend on cultivar and harvest year*, Food Chemistry. Elsevier Ltd, 122(3), pp. 819–825. doi: 10.1016/j.foodchem.2010.03.068.

Bonilla, J., Vargas, M., Atarés, L. and Chiralt, A. (2014) *Effect of Chitosan Essential Oil Films on the Storage-Keeping Quality of Pork Meat Products*, Food and Bioprocess Technology. Springer New York LLC, 7(8), pp. 2443–2450. doi: 10.1007/s11947-014-1329-3.

Bordes, P., Pollet, E. and Avérous, L. (2009) *Nano-biocomposites: Biodegradable polyester/nanoclay systems*, Progress in Polymer Science (Oxford). Pergamon, pp. 125–155. doi: 10.1016/j.progpolymsci.2008.10.002.

Bourbigot, S. and Fontaine, G. (2010) *Flame retardancy of polylactide: An overview*, Polymer Chemistry. The Royal Society of Chemistry, pp. 1413–1422. doi: 10.1039/c0py00106f.

Braun, B., Dorgan, J. R. and Knauss, D. M. (2006) *Reactively compatibilized cellulosic polylactide microcomposites*, Journal of Polymers and the Environment. Springer, 14(1), pp. 49–58. doi: 10.1007/s10924-005-8706-y.

Brzeziński, M. and Biela, T. (2014) *Polylactide nanocomposites with functionalized carbon nanotubes and their stereocomplexes: A focused review*, Materials Letters.

Elsevier B.V., 121, pp. 244–250. doi: 10.1016/j.matlet.2014.01.159.

De Camargo, A. C., Vidal, C. M. M., Canniatti-Brazaca, S. G. and Shahidi, F. (2014) *Fortification of cookies with peanut skins: Effects on the composition, polyphenols, antioxidant properties, and sensory quality*, Journal of Agricultural and Food Chemistry. American Chemical Society, 62(46), pp. 11228–11235. doi: 10.1021/jf503625p.

Carrasco, F., Pagès, P., Gámez-Pérez, J., Santana, O. O. and MasPOCH, M. L. (2010) *Processing of poly(lactic acid): Characterization of chemical structure, thermal stability and mechanical properties*, Polymer Degradation and Stability, 95(2), pp. 116–125. doi: 10.1016/j.polymdegradstab.2009.11.045.

Castro-Aguirre, E., Iñiguez-Franco, F., Samsudin, H., Fang, X. and Auras, R. (2016) *Poly(lactic acid)-Mass production, processing, industrial applications, and end of life*, Advanced Drug Delivery Reviews. Elsevier B.V., 107, pp. 333–366. doi: 10.1016/j.addr.2016.03.010.

Chambi, H. and Grosso, C. (2006) *Edible films produced with gelatin and casein cross-linked with transglutaminase*, Food Research International. Elsevier, 39(4), pp. 458–466. doi: 10.1016/j.foodres.2005.09.009.

Chang, S. K., Alasalvar, C., Bolling, B. W. and Shahidi, F. (2016) *Nuts and their co-products: The impact of processing (roasting) on phenolics, bioavailability, and health benefits - A comprehensive review*, Journal of Functional Foods. Elsevier Ltd, 26, pp. 88–122. doi: 10.1016/j.jff.2016.06.029.

Chen, C. Y. O. and Blumberg, J. B. (2008) *In vitro activity of almond skin polyphenols for scavenging free radicals and inducing quinone reductase*, Journal of Agricultural and Food Chemistry. J Agric Food Chem, 56(12), pp. 4427–4434. doi: 10.1021/jf800061z.

Crowe, T. D. and White, P. J. (2001) *Adaptation of the AOCS official method for measuring hydroperoxides from small-scale oil samples*, JAOCS, Journal of the American Oil Chemists' Society, 78(12), pp. 1267–1269. doi: 10.1007/s11745-001-0424-7.

Darie-Niță, R. N., Vasile, C., Stoleru, E., Pamfil, D., Zaharescu, T., Tarțău, L., Tudorachi, N., Brebu, M. A., Pricope, G. M., Dumitriu, R. P. and Leluk, K. (2018)

Evaluation of the rosemary extract effect on the properties of polylactic acid-based materials, *Materials*, 11(10). doi: 10.3390/ma11101825.

Datta, R. and Henry, M. (2006) *Lactic acid: recent advances in products, processes and technologies – a review*, *Journal of Chemical Technology and Biotechnology*, 81, pp. 1119–1129.

Datta, R., Tsai, S., Bonsignore, P., Moon, S. and Frank, J. R. (1995) *Technological and economical potential of poly(lactic acid) and lactic acid derivatives*, *FEMS Microbiology Reviews*, 16, pp. 221–231.

Dhar, P., Tarafder, D., Kumar, A. and Katiyar, V. (2015) *Effect of cellulose nanocrystal polymorphs on mechanical, barrier and thermal properties of poly(lactic acid) based bionanocomposites*, *RSC Advances*. Royal Society of Chemistry, 5(74), pp. 60426–60440. doi: 10.1039/c5ra06840a.

Domínguez-Robles, J., Martín, N. K., Fong, M. L., Stewart, S. A., Irwin, N. J., Rial-Hermida, M. I., Donnelly, R. F. and Larrañeta, E. (2019) *Antioxidant PLA composites containing lignin for 3D printing applications: A potential material for healthcare applications*, *Pharmaceutics*, 11(4), pp. 5–7. doi: 10.3390/pharmaceutics11040165.

Dorgan, J. R., Janzen, J., Clayton, M. P., Hait, S. B. and Knauss, D. M. (2005) *Melt rheology of variable L -content poly(lactic acid)*, *Journal of Rheology*, 49(3), pp. 607–619. doi: 10.1122/1.1896957.

Dorgan, J. R., Lehermeier, H. J., Palade, L.-I. and Cicero, J. (2001) *Poly lactides: Properties and Prospects of an Environmentally Benign Plastic from Renewable Resources*, *Macromol. Symp.*, 175, pp. 55–66.

Enayat, S. and Banerjee, S. (2009) *Comparative antioxidant activity of extracts from leaves, bark and catkins of Salix aegyptiaca sp.*, *Food Chemistry*. Elsevier, 116(1), pp. 23–28. doi: 10.1016/j.foodchem.2009.01.092.

Erdohan, Z. Ö., Çam, B. and Turhan, K. N. (2013) *Characterization of antimicrobial polylactic acid based films*, *Journal of Food Engineering*, 119(2), pp. 308–315. doi: 10.1016/j.jfoodeng.2013.05.043.

FAOSTAT (2019) *FAOSTAT, Food and Agriculture Organization of the United Nations* [Online]. Available at: <http://www.fao.org/faostat/en/#data/QC/visualize>

(Accessed: 30 June 2021).

Farah, S., Anderson, D. G. and Langer, R. (2016) *Physical and mechanical properties of PLA, and their functions in widespread applications -A comprehensive review*, *Advanced Drug Delivery Reviews*. Elsevier B.V., 107, pp. 367–392. doi: 10.1016/j.addr.2016.06.012.

Farrington, D. W., Lunt, J., Davies, S. and Blackburn, R. S. (2005) *Poly(lactic acid) fibers*, *Biodegradable and Sustainable Fibres*. Elsevier. doi: 10.1533/9781845690991.191.

Faruk, O., Bledzki, A. K., Fink, H. P. and Sain, M. (2012) *Biocomposites reinforced with natural fibers: 2000-2010*, *Progress in Polymer Science*. Pergamon, pp. 1552–1596. doi: 10.1016/j.progpolymsci.2012.04.003.

Fasihnia, S. H., Peighamardoust, S. H., Peighamardoust, S. J., Oromiehie, A., Soltanzadeh, M. and Peressini, D. (2020) *Migration analysis, antioxidant, and mechanical characterization of polypropylene-based active food packaging films loaded with BHA, BHT, and TBHQ*, *Journal of Food Science*, 85(8), pp. 2317–2328. doi: 10.1111/1750-3841.15337.

Fox, D. M., Lee, J., Zammarano, M., Katsoulis, D., Eldred, D. V., Haverhals, L. M., Trulove, P. C., De Long, H. C. and Gilman, J. W. (2012) *Char-forming behavior of nanofibrillated cellulose treated with glycidyl phenyl POSS*, *Carbohydrate Polymers*. Elsevier, 88(3), pp. 847–858. doi: 10.1016/j.carbpol.2012.01.015.

Frone, A. N., Berlioz, S., Chailan, J.-F., Panaitescu, D. M. and Donescu, D. (2011) *Cellulose fiber-reinforced polylactic acid*, *Polymer Composites*. John Wiley & Sons, Ltd, 32(6), pp. 976–985. doi: 10.1002/pc.21116.

Fukushima, K., Murariu, M., Camino, G. and Dubois, P. (2010) *Effect of expanded graphite/layered-silicate clay on thermal, mechanical and fire retardant properties of poly(lactic acid)*, *Polymer Degradation and Stability*. Elsevier, 95(6), pp. 1063–1076. doi: 10.1016/j.polymdegradstab.2010.02.029.

García, A. V., Serrano, N. J., Sanahuja, A. B. and Garrigós, M. C. (2020) *Novel antioxidant packaging films based on poly(ϵ -caprolactone) and almond skin extract: Development and effect on the oxidative stability of fried almonds*, *Antioxidants*, 9(7), pp. 1–18. doi: 10.3390/antiox9070629.

Garlotta, D. (2019) *A Literature Review of Poly (Lactic Acid)*, Journal of Polymers and the Environment, 9(2), pp. 63–84.

Garrido, I., Monagas, M., Gómez-Cordovés, C. and Bartolomé, B. (2008a) *Polyphenols and antioxidant properties of almond skins: Influence of industrial processing*, Journal of Food Science. John Wiley & Sons, Ltd, 73(2), pp. C106–C115. doi: 10.1111/j.1750-3841.2007.00637.x.

Garrido, I., Monagas, M., Gómez-Cordovés, C. and Bartolomé, B. (2008b) *Polyphenols and Antioxidant Properties of Almond Skins: Influence of Industrial Processing*, Journal of Food Science. John Wiley & Sons, Ltd, 73(2), pp. C106–C115. doi: 10.1111/j.1750-3841.2007.00637.x.

Giita Silverajah, V. S., Ibrahim, N. A., Zainuddin, N., Wan Yunus, W. M. Z. and Hassan, H. A. (2012) *Mechanical, thermal and morphological properties of poly(lactic acid)/epoxidized palm olein blend*, Molecules, 17(10), pp. 11729–11747. doi: 10.3390/molecules171011729.

Gonçalves, C. M. B., Tomé, L. C., Coutinho, J. A. P. and Marrucho, I. M. (2011) *Addition of α -tocopherol on poly(lactic acid): Thermal, mechanical, and sorption properties*, Journal of Applied Polymer Science, 119(4), pp. 2468–2475. doi: 10.1002/app.32952.

Gordon, M. H. (2004) *Understanding and Measuring the Shelf-Life of Food / ScienceDirect*, Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 128–141. Available at: <https://www.sciencedirect.com/book/9781855737327/understanding-and-measuring-the-shelf-life-of-food> (Accessed: 4 July 2021).

Gradziel, T. M. (2009) *Almond (Prunus dulcis) breeding*, Breeding Plantation Tree Crops: Temperate Species. Springer New York, pp. 1–31. doi: 10.1007/978-0-387-71203-1_1.

Graupner, N., Herrmann, A. S. and Müssig, J. (2009) *Natural and man-made cellulose fibre-reinforced poly(lactic acid) (PLA) composites: An overview about mechanical characteristics and application areas*, Composites Part A: Applied Science and Manufacturing. Elsevier, 40(6–7), pp. 810–821. doi: 10.1016/j.compositesa.2009.04.003.

Gültekin, N., Tihminlioğlu, F., Çiftçioğlu, R., Çiftçioğlu, M. and Harsa, Ş. (2004) *Preparation and characterization of polyLactide-hydroxyapatite biocomposites*, Key Engineering Materials. Trans Tech Publications Ltd, pp. 1953–1956. doi: 10.4028/www.scientific.net/kem.264-268.1953.

Gupta, B., Revagade, N. and Hilborn, J. (2007) *Poly(lactic acid) fiber: An overview*, Progress in Polymer Science (Oxford), 32(4), pp. 455–482. doi: 10.1016/j.progpolymsci.2007.01.005.

Hamad, K., Kaseem, M., Yang, H. W., Deri, F. and Ko, Y. G. (2015) *Properties and medical applications of polylactic acid: A review*, Express Polymer Letters, 9(5), pp. 435–455. doi: 10.3144/expresspolymlett.2015.42.

Han, J. H. and Floros, J. D. (1997) *Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity*, Journal of Plastic Film and Sheeting, 13(4), pp. 287–298. doi: 10.1177/875608799701300405.

Harris, A. M. and Lee, E. C. (2008) *Improving mechanical performance of injection molded PLA by controlling crystallinity*, Journal of Applied Polymer Science. John Wiley & Sons, Ltd, 107(4), pp. 2246–2255. doi: 10.1002/app.27261.

Hashemian, S., Salari, K. and Yazdi, Z. A. (2014) *Journal of Industrial and Engineering Chemistry Preparation of activated carbon from agricultural wastes (almond shell and orange peel) for adsorption of 2-pic from aqueous solution*, Journal of Industrial and Engineering Chemistry. The Korean Society of Industrial and Engineering Chemistry, 20(4), pp. 1892–1900. doi: 10.1016/j.jiec.2013.09.009.

Henton, D. E., Gruber, P., Lunt, J. and Randall, J. (2005) *Polylactic acid technology*, Natural Fibers, Biopolymers, and Biocomposites, 48674(23), pp. 527–577. doi: 10.1002/1521-4095(200012)12:23<1841::aid-adma1841>3.3.co;2-5.

Huang, Y., Wang, T., Zhao, X., Wang, X., Zhou, L., Yang, Y., Liao, F. and Ju, Y. (2015) *Poly(lactic acid)/graphene oxide-ZnO nanocomposite films with good mechanical, dynamic mechanical, anti-UV and antibacterial properties*, Journal of Chemical Technology & Biotechnology. John Wiley and Sons Ltd, 90(9), pp. 1677–1684. doi: 10.1002/jctb.4476.

Hwang, S. W., Shim, J. K., Selke, S. E., Soto-Valdez, H., Matuana, L., Rubino, M. and Auras, R. (2012) *Poly(lactic acid) with added α -tocopherol and resveratrol:*

optical, physical, thermal and mechanical properties, Polymer International. John Wiley & Sons, Ltd, 61(3), pp. 418–425. doi: 10.1002/pi.3232.

Inkinen, S., Hakkarainen, M., Albertsson, A. C. and Södergård, A. (2011) *From lactic acid to poly(lactic acid) (PLA): Characterization and analysis of PLA and Its precursors*, Biomacromolecules, 12(3), pp. 523–532. doi: 10.1021/bm101302t.

Jamshidian, M., Arab Tehrany, E., Cleymand, F., Leconte, S., Falher, T. and Desobry, S. (2012) *Effects of synthetic phenolic antioxidants on physical, structural, mechanical and barrier properties of poly lactic acid film*, Carbohydrate Polymers. Elsevier Ltd., 87(2), pp. 1763–1773. doi: 10.1016/j.carbpol.2011.09.089.

Jamshidian, M., Tehrany, E. A., Imran, M., Akhtar, M. J., Cleymand, F. and Desobry, S. (2012) *Structural, mechanical and barrier properties of active PLA-antioxidant films*, Journal of Food Engineering. Elsevier Ltd, 110(3), pp. 380–389. doi: 10.1016/j.jfoodeng.2011.12.034.

Jamshidian, M., Tehrany, E. A., Imran, M., Jacquot, M. and Desobry, S. (2010) *Poly-Lactic Acid: Production, applications, nanocomposites, and release studies*, Comprehensive Reviews in Food Science and Food Safety, 9(5), pp. 552–571. doi: 10.1111/j.1541-4337.2010.00126.x.

V. Janorkar, A., T. Metters, A. and E. Hirt, D. (2004) *Modification of Poly(lactic acid) Films: Enhanced Wettability from Surface-Confined Photografting and Increased Degradation Rate Due to an Artifact of the Photografting Process*, Macromolecules, 37(24), pp. 9151–9159. doi: 10.1021/ma049056u.

John, M. J. and Thomas, S. (2008) *Biofibres and biocomposites*, Carbohydrate Polymers. Elsevier, pp. 343–364. doi: 10.1016/j.carbpol.2007.05.040.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. (2008) *Antioxidative activity and properties of fish skin gelatin films incorporated with BHT and α -tocopherol*, Food Hydrocolloids, 22(3), pp. 449–458. doi: 10.1016/j.foodhyd.2007.01.002.

Julien, J. M., Bénézet, J. C., Lafranche, E., Quantin, J. C., Bergeret, A., Lacrampe, M. F. and Krawczak, P. (2012) *Development of poly(lactic acid) cellular materials: Physical and morphological characterizations*, Polymer. Elsevier Ltd, 53(25), pp. 5885–5895. doi: 10.1016/j.polymer.2012.10.005.

Kanik, T. and Kabak, B. (2019) *Aflatoxins in almonds: Monitoring and exposure assessment*, Journal of Food Safety. Blackwell Publishing Ltd, 39(4). doi: 10.1111/jfs.12646.

Kanmani, P. and Rhim, J. W. (2014) *Antimicrobial and physical-mechanical properties of agar-based films incorporated with grapefruit seed extract*, Carbohydrate Polymers. Elsevier Ltd., 102(1), pp. 708–716. doi: 10.1016/j.carbpol.2013.10.099.

Khang, G. (2013) *Drug Delivery System Using Polymers (Lactide–Co-Glycolide)*, IFMBE Proceedings. Springer, Berlin, Heidelberg, 40 IFMBE, pp. 258–261. doi: 10.1007/978-3-642-32183-2_65.

Kuden, A. B., Kuden, A., Bayait, S., Comlekcioglu, S., Imrak, B. and Rehber Dikkaya, Y. (2014) *Badem Yetiştiriciliği, Eylül, 2014*, Tagep Proje No.: 5.2.3.1. Available at: <https://azkurs.org/badem-yetistiriciligi-eylul-2014-tagep-proje-no-2-1.html> (Accessed: 24 January 2021).

Kulinski, Z. and Piorkowska, E. (2005a) *Crystallization, structure and properties of plasticized poly(L-lactide)*, Polymer, 46(23), pp. 10290–10300. doi: 10.1016/j.polymer.2005.07.101.

Kulinski, Z. and Piorkowska, E. (2005b) *Crystallization, structure and properties of plasticized poly(L-lactide)*, Polymer. Elsevier BV, 46(23), pp. 10290–10300. doi: 10.1016/j.polymer.2005.07.101.

Kulkarni, R. K., Moore, E. G., Hegyeli, A. F. and Leonard, F. (1971) *Biodegradable poly(lactic acid) polymers*, Journal of Biomedical Materials Research. John Wiley & Sons, Ltd, 5(3), pp. 169–181. doi: 10.1002/JBM.820050305.

Kurt, A. and Kahyaoglu, T. (2014) *Characterization of a new biodegradable edible film made from salep glucomannan*, Carbohydrate Polymers. Elsevier, 104(1), pp. 50–58. doi: 10.1016/j.carbpol.2014.01.003.

Lalla, J. K. and Chugh, N. N. (1990) *Biodegradable polymer polylactic acid. Part I: Synthesis evaluation and structure elucidation*, Indian Drugs, 27(10), pp. 516–522.

Lasprilla, A. J. R., Martinez, G. A. R., Lunelli, B. H., Jardini, A. L. and Filho, R. M. (2012) *Poly-lactic acid synthesis for application in biomedical devices - A review*,

Biotechnology Advances. Elsevier Inc., 30(1), pp. 321–328. doi: 10.1016/j.biotechadv.2011.06.019.

Lee, S. Y., Kang, I. A., Doh, G. H., Yoon, H. G., Park, B. D. and Wu, Q. (2008) *Thermal and mechanical properties of wood flour/talc-filled polylactic acid composites: Effect of filler content and coupling treatment*, Journal of Thermoplastic Composite Materials, 21(3), pp. 209–223. doi: 10.1177/0892705708089473.

Li, L., Ding, S. and Zhou, C. (2004) *Preparation and degradation of PLA/chitosan composite materials*, Journal of Applied Polymer Science, 91(1). doi: 10.1002/app.12954.

Lim, L. T., Auras, R. and Rubino, M. (2008) *Processing technologies for poly(lactic acid)*, Progress in Polymer Science (Oxford), 33(8), pp. 820–852. doi: 10.1016/j.progpolymsci.2008.05.004.

Liu, H. and Zhang, J. (2011) *Research progress in toughening modification of poly(lactic acid)*, Journal of Polymer Science, Part B: Polymer Physics, 49(15), pp. 1051–1083. doi: 10.1002/polb.22283.

Liu, Y., Liang, X., Wang, S., Qin, W. and Zhang, Q. (2018) *Electrospun antimicrobial polylactic acid/tea polyphenol nanofibers for food-packaging applications*, Polymers, 10(5). doi: 10.3390/polym10050561.

Madhavan Nampoothiri, K., Nair, N. R. and John, R. P. (2010) *An overview of the recent developments in polylactide (PLA) research*, Bioresource Technology. Elsevier Ltd, 101(22), pp. 8493–8501. doi: 10.1016/j.biortech.2010.05.092.

Mandalari, G., Tomaino, A., Arcoraci, T., Martorana, M., Lo Turco, V., Cacciola, F., Rich, G. T., Bisignano, C., Saija, A., Dugo, P., Cross, K. L., Parker, M. L., Waldron, K. W. and Wickham, J. (2010) *Characterization of polyphenols, lipids and dietary fibre from almond skins (Amygdalus communis L.)*, Journal of Food Composition and Analysis, 23(2), pp. 166–174. doi: 10.1016/j.jfca.2009.08.015.

Mankotia, K., Singh, I. and Singh, R. (2020) *On effect of almond skin powder waste reinforcement in PA6: Rheological, thermal and wear properties*, Materials Today: Proceedings, pp. 3–8. doi: 10.1016/j.matpr.2020.03.812.

Manzanarez-López, F., Soto-Valdez, H., Auras, R. and Peralta, E. (2011) *Release of*

α-Tocopherol from Poly(lactic acid) films, and its effect on the oxidative stability of soybean oil, Journal of Food Engineering, 104(4), pp. 508–517. doi: 10.1016/j.jfoodeng.2010.12.029.

Martins, C., Vilarinho, F., Sanches Silva, A., Andrade, M., Machado, A. V., Castilho, M. C., Sá, A., Cunha, A., Vaz, M. F. and Ramos, F. (2018) *Active polylactic acid film incorporated with green tea extract: Development, characterization and effectiveness*, Industrial Crops and Products, 123(February), pp. 100–110. doi: 10.1016/j.indcrop.2018.06.056.

Meshkini, A. (2016) *Acetone Extract of Almond Hulls Provides Protection against Oxidative Damage and Membrane Protein Degradation*, JAMS Journal of Acupuncture and Meridian Studies. Elsevier Korea LLC, 9(3), pp. 134–142. doi: 10.1016/j.jams.2015.10.001.

Milbury, P. E., Chen, C. Y., Dolnikowski, G. G. and Blumberg, J. B. (2006) *Determination of flavonoids and phenolics and their distribution in almonds*, Journal of Agricultural and Food Chemistry, 54(14), pp. 5027–5033. doi: 10.1021/jf0603937.

Moghadam, M., Salami, M., Mohammadian, M., Khodadadi, M. and Emam-Djomeh, Z. (2020) *Development of antioxidant edible films based on mung bean protein enriched with pomegranate peel*, Food Hydrocolloids. Elsevier B.V., 104, p. 105735. doi: 10.1016/j.foodhyd.2020.105735.

Monagas, M., Garrido, I., Lebrón-Aguilar, R., Bartolome, B. and Gómez-Cordovés, C. (2007) *Almond (*Prunus dulcis* (Mill.) D.A. Webb) skins as a potential source of bioactive polyphenols*, Journal of Agricultural and Food Chemistry, 55(21), pp. 8498–8507. doi: 10.1021/jf071780z.

Monagas, M., Garrido, I., Lebrón-Aguilar, R., Carmen Gómez-Cordovés, M., Rybarczyk, A., Amarowicz, R. and Bartolomé, B. (2009) *Comparative flavan-3-ol profile and antioxidant capacity of roasted peanut, hazelnut, and almond skins*, Journal of Agricultural and Food Chemistry. J Agric Food Chem, 57(22), pp. 10590–10599. doi: 10.1021/jf901391a.

Moo-Huchin, V. M., Moo-Huchin, M. I., Estrada-León, R. J., Cuevas-Glory, L., Estrada-Mota, I. A., Ortiz-Vázquez, E., Betancur-Ancona, D. and Sauri-Duch, E. (2015) *Antioxidant compounds, antioxidant activity and phenolic content in peel from*

three tropical fruits from Yucatan, Mexico, Food Chemistry, 166, pp. 17–22. doi: 10.1016/j.foodchem.2014.05.127.

Motedayen, A. A., Khodaiyan, F. and Salehi, E. A. (2013) *Development and characterisation of composite films made of kefirin and starch*, Food Chemistry. Elsevier Ltd, 136(3–4), pp. 1231–1238. doi: 10.1016/j.foodchem.2012.08.073.

Mukherjee, T. and Kao, N. (2011) *PLA Based Biopolymer Reinforced with Natural Fibre: A Review*, Journal of Polymers and the Environment. Springer, 19(3), pp. 714–725. doi: 10.1007/s10924-011-0320-6.

Murariu, M., Dechief, A. L., Bonnaud, L., Paint, Y., Gallos, A., Fontaine, G., Bourbigot, S. and Dubois, P. (2010) *The production and properties of polylactide composites filled with expanded graphite*, Polymer Degradation and Stability. Elsevier, 95(5), pp. 889–900. doi: 10.1016/j.polymdegradstab.2009.12.019.

Murariu, M., Doumbia, A., Bonnaud, L., Dechief, A. L., Paint, Y., Ferreira, M., Campagne, C., Devaux, E. and Dubois, P. (2011) *High-performance polylactide/ZnO nanocomposites designed for films and fibers with special end-use properties*, Biomacromolecules. American Chemical Society, 12(5), pp. 1762–1771. doi: 10.1021/bm2001445.

Murariu, M. and Dubois, P. (2016) *PLA composites: From production to properties*, Advanced Drug Delivery Reviews. Elsevier B.V., 107, pp. 17–46. doi: 10.1016/j.addr.2016.04.003.

Murariu, M., Laoutid, F., Dubois, P., Fontaine, G., Bourbigot, S., Devaux, E., Campagne, C., Ferreira, M. and Solarski, S. (2014) *Pathways to Biodegradable Flame Retardant Polymer (Nano)Composites*, Polymer Green Flame Retardants. Elsevier Inc., pp. 709–773. doi: 10.1016/B978-0-444-53808-6.00021-4.

Nagarajan, V., Mohanty, A. K. and Misra, M. (2016) *Perspective on Polylactic Acid (PLA) based Sustainable Materials for Durable Applications: Focus on Toughness and Heat Resistance*, ACS Sustainable Chemistry and Engineering, 4(6), pp. 2899–2916. doi: 10.1021/acssuschemeng.6b00321.

Nasirahmadi, A. and Miraei Ashtiani, S. H. (2017) *Bag-of-Feature model for sweet and bitter almond classification*, Biosystems Engineering. Academic Press, 156, pp. 51–60. doi: 10.1016/j.biosystemseng.2017.01.008.

Norajit, K., Kim, K. M. and Ryu, G. H. (2010) *Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract*, Journal of Food Engineering. Elsevier Ltd, 98(3), pp. 377–384. doi: 10.1016/j.jfoodeng.2010.01.015.

Oliveira, R. N., Mancini, M. C., de Oliveira, F. C. S., Passos, T. M., Quilty, B., Thiré, R. M. da S. M. and McGuinness, G. B. (2016) *Análise por FTIR e quantificação de fenóis e flavonóides de cinco produtos naturais disponíveis comercialmente utilizados no tratamento de feridas*, Revista Materia, 21(3), pp. 767–779. doi: 10.1590/S1517-707620160003.0072.

Omeroğlu Ay, Ç., Ozcan, A. S., Erdoğan, Y. and Ozcan, A. (2012) *Characterization of Punica granatum L. peels and quantitatively determination of its biosorption behavior towards lead(II) ions and Acid Blue 40*, Colloids and Surfaces B: Biointerfaces, 100, pp. 197–204. doi: 10.1016/j.colsurfb.2012.05.013.

Ouchiar, S., Stoclet, G., Cabaret, C., Georges, E., Smith, A., Martias, C., Addad, A. and Gloaguen, V. (2015) *Comparison of the influence of talc and kaolinite as inorganic fillers on morphology, structure and thermomechanical properties of polylactide based composites*, Applied Clay Science. Elsevier Ltd, 116–117, pp. 231–240. doi: 10.1016/j.clay.2015.03.020.

Ozdemir, K. S., Yilmaz, C., Durmaz, G. and Gokmen, V. (2014) *Hazelnut skin powder: A new brown colored functional ingredient*, Food Research International. Elsevier Ltd, 65(PB), pp. 291–297. doi: 10.1016/j.foodres.2014.01.060.

P. Pawar, R., U. Tekale, S., U. Shisodia, S., T. Totre, J. and J. Domb, A. (2014) *Biomedical Applications of Poly(Lactic Acid)*, Recent Patents on Regenerative Medicine, 4(1). doi: 10.2174/2210296504666140402235024.

Pang, X., Zhuang, X., Tang, Z. and Chen, X. (2010) *Poly(lactic acid) (PLA): Research, development and industrialization*, Biotechnology Journal, 5(11), pp. 1125–1136. doi: 10.1002/biot.201000135.

Pastor, C., Sánchez-González, L., Chiralt, A., Cháfer, M. and González-Martínez, C. (2013) *Physical and antioxidant properties of chitosan and methylcellulose based films containing resveratrol*, Food Hydrocolloids. Elsevier, 30(1), pp. 272–280. doi: 10.1016/j.foodhyd.2012.05.026.

Perego, G., Cella, G. D. and Bastioli, C. (1996) *Effect of molecular weight and crystallinity on poly(lactic acid) mechanical properties*, Journal of Applied Polymer Science, 59(1), pp. 37–43. doi: 10.1002/(sici)1097-4628(19960103)59:1<37::aid-app6>3.0.co;2-n.

Pickering, K. L., Efendy, M. G. A. and Le, T. M. (2016) *A review of recent developments in natural fibre composites and their mechanical performance*, Composites Part A: Applied Science and Manufacturing. Elsevier Ltd, pp. 98–112. doi: 10.1016/j.compositesa.2015.08.038.

Pilla, S., Gong, S., O'Neill, E., Yang, L. and Rowell, R. M. (2009) *Poly lactide-recycled wood fiber composites*, Journal of Applied Polymer Science. John Wiley & Sons, Ltd, 111(1), pp. 37–47. doi: 10.1002/app.28860.

Piorkowska, E., Kulinski, Z., Galeski, A. and Masirek, R. (2006) *Plasticization of semicrystalline poly(l-lactide) with poly(propylene glycol)*, Polymer. Elsevier BV, 47(20), pp. 7178–7188. doi: 10.1016/j.polymer.2006.03.115.

Prgomet, I., Goncalves, B., Domínguez-Perles, R., Pascual-Seva, N. and Barros, A. I. R. N. A. (2017) *Valorization challenges to almond residues: Phytochemical composition and functional application*, Molecules, 22(10). doi: 10.3390/molecules22101774.

Prgomet, I., Gonçalves, B., Domínguez-Perles, R., Pascual-Seva, N. and Barros, A. I. R. N. A. (2019) *A Box-Behnken Design for Optimal Extraction of Phenolics from Almond By-products*, Food Analytical Methods. Springer New York LLC, 12(9), pp. 2009–2024. doi: 10.1007/s12161-019-01540-5.

Puaux, J. P., Banu, I., Nagy, I. and Bozga, G. (2007) *A study of L-lactide ring-opening polymerization kinetics*, Macromolecular Symposia, 259, pp. 318–326. doi: 10.1002/masy.200751336.

[Quora]. (2020, March 25). [Web based image]. Available at: <https://www.quora.com/Why-are-you-supposed-to-soak-almonds-in-water-and-peel-the-skins-off-before-eating-them-What-is-the-benefit-of-doing-this-Can-we-eat-them-without-peeling-the-skin-Is-eating-the-skin-harmful>

Radusin, T., Tomšik, A., Šarić, L., Ristić, I., Giacinti Baschetti, M., Minelli, M. and Novaković, A. (2019) *Hybrid Pla/wild garlic antimicrobial composite films for food*

packaging application, Polymer Composites. John Wiley and Sons Inc., 40(3), pp. 893–900. doi: 10.1002/pc.24755.

Rai, S., Dutta, P. K. and Mehrotra, G. K. (2020) *Natural Antioxidant and Antimicrobial Agents from Agrowastes: An Emergent Need to Food Packaging*, Waste and Biomass Valorization. Springer Netherlands, 11(5), pp. 1905–1916. doi: 10.1007/s12649-018-0498-0.

Ramamoorthy, S. K., Skrifvars, M. and Persson, A. (2015) *A Review of Natural Fibers Used in Biocomposites: Plant, Animal and Regenerated Cellulose Fibers*, Polymer Reviews. Taylor and Francis Inc., 55(1), pp. 107–162. doi: 10.1080/15583724.2014.971124.

Raquez, J. M., Habibi, Y., Murariu, M. and Dubois, P. (2013) *Poly(lactide (PLA)-based nanocomposites*, Progress in Polymer Science. Elsevier Ltd, 38(10–11), pp. 1504–1542. doi: 10.1016/j.progpolymsci.2013.05.014.

Rasal, R. M., Janorkar, A. V. and Hirt, D. E. (2010) *Poly(lactic acid) modifications*, Progress in Polymer Science (Oxford). Elsevier Ltd, 35(3), pp. 338–356. doi: 10.1016/j.progpolymsci.2009.12.003.

[RawFoodLife]. (2019, April 3). Discover the Science of Raw Food [Web based image]. Available at: <https://rawfoodlife.com/product/capay-hills-certified-organic-almonds/>

Riaz, A., Lagnika, C., Luo, H., Dai, Z., Nie, M., Hashim, M. M., Liu, C., Song, J. and Li, D. (2020) *Chitosan-based biodegradable active food packaging film containing Chinese chive (*Allium tuberosum*) root extract for food application*, International Journal of Biological Macromolecules. Elsevier B.V., 150, pp. 595–604. doi: 10.1016/j.ijbiomac.2020.02.078.

Saeidlou, S., Huneault, M. A., Li, H. and Park, C. B. (2012) *Poly(lactic acid) crystallization*, Progress in Polymer Science. Elsevier Ltd, 37(12), pp. 1657–1677. doi: 10.1016/j.progpolymsci.2012.07.005.

Salević, A., Prieto, C., Cabedo, L., Nedović, V. and Lagaron, J. M. (2019) *Physicochemical, antioxidant and antimicrobial properties of electrospun poly(ϵ -caprolactone) films containing a solid dispersion of sage (*Salvia officinalis* L.) extract*, Nanomaterials, 9(2), pp. 1–18. doi: 10.3390/nano9020270.

Samsudin, H., Soto-Valdez, H. and Auras, R. (2014) *Poly(lactic acid) film incorporated with marigold flower extract (Tagetes erecta) intended for fatty-food application*, Food Control. Elsevier Ltd, 46, pp. 55–66. doi: 10.1016/j.foodcont.2014.04.045.

Sang, S., Lapsley, K., Jeong, W. S., Lachance, P. A., Ho, C. T. and Rosen, R. T. (2002) *Antioxidative phenolic compounds isolated from almond skins (Prunus amygdalus Batsch)*, Journal of Agricultural and Food Chemistry. J Agric Food Chem, 50(8), pp. 2459–2463. doi: 10.1021/jf011533+.

Saricaoglu, F. T., Tural, S., Gul, O. and Turhan, S. (2018) *High pressure homogenization of mechanically deboned chicken meat protein suspensions to improve mechanical and barrier properties of edible films*, Food Hydrocolloids. Elsevier Ltd, 84, pp. 135–145. doi: 10.1016/j.foodhyd.2018.05.058.

Shahidi, F., Alasalvar, C. and Liyana-Pathirana, C. M. (2007) *Antioxidant phytochemicals in hazelnut kernel (Corylus avellana L) and hazelnut byproducts*, Journal of Agricultural and Food Chemistry. J Agric Food Chem, 55(4), pp. 1212–1220. doi: 10.1021/jf062472o.

Shakoor, A. and Thomas, N. L. (2014) *Talc as a nucleating agent and reinforcing filler in poly(lactic acid) composites*, Polymer Engineering & Science. John Wiley & Sons, Ltd, 54(1), pp. 64–70. doi: 10.1002/pen.23543.

Shankar, S. and Rhim, J. W. (2018) *Preparation of antibacterial poly(lactide)/poly(butylene adipate-co-terephthalate) composite films incorporated with grapefruit seed extract*, International Journal of Biological Macromolecules. Elsevier B.V., 120, pp. 846–852. doi: 10.1016/j.ijbiomac.2018.09.004.

da Silva, C., Prasniewski, A., Calegari, M. A., de Lima, V. A. and Oldoni, T. L. C. (2018) *Determination of Total Phenolic Compounds and Antioxidant Activity of Ethanolic Extracts of Propolis Using ATR-FT-IR Spectroscopy and Chemometrics*, Food Analytical Methods, 11(7), pp. 2013–2021. doi: 10.1007/s12161-018-1161-x.

Singh, R., Kumar, R., Singh, M. and Preet, P. (2020) *On compressive and morphological features of 3D printed almond skin powder reinforced PLA matrix*, Materials Research Express. IOP Publishing, 7(2). doi: 10.1088/2053-1591/ab5e61.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. (1999) *Analysis of total*

phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, *Methods in Enzymology*. Academic Press Inc., 299, pp. 152–178. doi: 10.1016/S0076-6879(99)99017-1.

Siqueira, G., Bras, J. and Dufresne, A. (2010) *Cellulosic Bionanocomposites: A Review of Preparation, Properties and Applications*, *Polymers. Molecular Diversity Preservation International*, 2(4), pp. 728–765. doi: 10.3390/polym2040728.

Siripatrawan, U. and Harte, B. R. (2010) *Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract*, *Food Hydrocolloids*. Elsevier Ltd, 24(8), pp. 770–775. doi: 10.1016/j.foodhyd.2010.04.003.

Smeriglio, A., Mandalari, G., Bisignano, C., Filocamo, A., Barreca, D., Bellocco, E. and Trombetta, D. (2016) *Polyphenolic content and biological properties of Avola almond (*Prunus dulcis* Mill. D.A. Webb) skin and its industrial byproducts*, *Industrial Crops and Products*. Elsevier B.V., 83, pp. 283–293. doi: 10.1016/j.indcrop.2015.11.089.

Socias I Company, R., Gómez Aparisi, J. and Alonso, J. M. (2005) *Year and enclosure effects on fruit set in an autogamous almond*, *Scientia Horticulturae*. Elsevier, 104(3), pp. 369–377. doi: 10.1016/j.scienta.2004.10.001.

Stoll, L., Rech, R., Flôres, S. H., Nachtigall, S. M. B. and de Oliveira Rios, A. (2018) *Carotenoids extracts as natural colorants in poly(lactic acid) films*, *Journal of Applied Polymer Science*. John Wiley and Sons Inc., 135(33), p. 46585. doi: 10.1002/app.46585.

Sun, L., Sun, J., Chen, L., Niu, P., Yang, X. and Guo, Y. (2017) *Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material*, *Carbohydrate Polymers*. Elsevier Ltd, 163, pp. 81–91. doi: 10.1016/j.carbpol.2017.01.016.

Taş, N. G. and Gökmen, V. (2017) *‘Phenolic compounds in natural and roasted nuts and their skins: a brief review’*, *Current Opinion in Food Science*, 14, pp. 103–109. doi: 10.1016/j.cofs.2017.03.001.

Thellen, C., Orroth, C., Froio, D., Ziegler, D., Lucciarini, J., Farrell, R., D’Souza, N. A. and Ratto, J. A. (2005) *Influence of montmorillonite layered silicate on plasticized poly(l-lactide) blown films*, *Polymer*, 46(25), pp. 11716–11727. doi:

10.1016/j.polymer.2005.09.057.

Tokiwa, Y. and Calabia, B. P. (2006) *Biodegradability and biodegradation of poly(lactide)*, Applied Microbiology and Biotechnology, 72(2), pp. 244–251. doi: 10.1007/s00253-006-0488-1.

Torres-Giner, S., Prieto, C. and Lagaron, J. M. (2020) *Nanomaterials to enhance food quality, safety, and health impact*, Nanomaterials. MDPI AG. doi: 10.3390/nano10050941.

Trifunsi, S., Munteanu, M. F., Agotici, V., Pinte, S. and Gligor, R. (2015) *Determination of Flavonoid and Polyphenol Compounds in Viscum Album and Allium Sativum Extracts*, International Current Pharmaceutical Journal, 4(5), pp. 382–385. doi: 10.3329/icpj.v4i5.22861.

Tsuji, H. (2005) *Poly(lactide) stereocomplexes: Formation, structure, properties, degradation, and applications*, Macromolecular Bioscience, 5(7), pp. 569–597. doi: 10.1002/mabi.200500062.

Valdés, A., Fenollar, O., Beltrán, A., Balart, R., Fortunati, E., Kenny, J. M. and Garrigós, M. C. (2016) *Characterization and enzymatic degradation study of poly(ϵ -caprolactone)-based biocomposites from almond agricultural by-products*, Polymer Degradation and Stability, 132, pp. 181–190. doi: 10.1016/j.polymdegradstab.2016.02.023.

Valdés, A., Mellinas, A. C., Ramos, M., Garrigós, M. C. and Jiménez, A. (2014) *Natural additives and agricultural wastes in biopolymer formulations for food packaging*, Frontiers in Chemistry, 2(FEB), pp. 1–10. doi: 10.3389/fchem.2014.00006.

Valdés, A., Vidal, L., Beltrán, A., Canals, A. and Garrigós, M. C. (2015) *Microwave-Assisted Extraction of Phenolic Compounds from Almond Skin Byproducts (Prunus amygdalus): A Multivariate Analysis Approach*, Journal of Agricultural and Food Chemistry, 63(22), pp. 5395–5402. doi: 10.1021/acs.jafc.5b01011.

Varzakas, T., Zakyntinos, G. and Verpoort, F. (2016) *Plant Food Residues as a Source of Nutraceuticals and Functional Foods*, Foods, 5(4), p. 88. doi: 10.3390/foods5040088.

Wang, L. F. and Rhim, J. W. (2016) *Grapefruit seed extract incorporated antimicrobial LDPE and PLA films: Effect of type of polymer matrix*, *LWT - Food Science and Technology*. Elsevier Ltd, 74, pp. 338–345. doi: 10.1016/j.lwt.2016.07.066.

Weng, Y. X., Jin, Y. J., Meng, Q. Y., Wang, L., Zhang, M. and Wang, Y. Z. (2013) *Biodegradation behavior of poly(butylene adipate-co-terephthalate) (PBAT), poly(lactic acid) (PLA), and their blend under soil conditions*, *Polymer Testing*. Elsevier Ltd, 32(5), pp. 918–926. doi: 10.1016/j.polymertesting.2013.05.001.

Wijeratne, S. S. K., Abou-Zaid, M. M. and Shahidi, F. (2006) *Antioxidant polyphenols in almond and its coproducts*, *Journal of Agricultural and Food Chemistry*, 54(2), pp. 312–318. doi: 10.1021/jf051692j.

Wijeratne, S. S. K., Amarowicz, R. and Shahidi, F. (2006) *Antioxidant activity of almonds and their by-products in food model systems*, *JAOCS, Journal of the American Oil Chemists' Society*, 83(3), pp. 223–230. doi: 10.1007/s11746-006-1197-8.

Wrona, M., Cran, M. J., Nerín, C. and Bigger, S. W. (2017) *Development and characterisation of HPMC films containing PLA nanoparticles loaded with green tea extract for food packaging applications*, *Carbohydrate Polymers*. Elsevier Ltd, 156, pp. 108–117. doi: 10.1016/j.carbpol.2016.08.094.

Wu, Y., Qin, Y., Yuan, M., Li, L., Chen, H., Cao, J. and Yang, J. (2014) *Characterization of an antimicrobial poly(lactic acid) film prepared with poly(ϵ -caprolactone) and thymol for active packaging*, *Polymers for Advanced Technologies*, 25(9), pp. 948–954. doi: 10.1002/pat.3332.

Www, W. :, Reddy, R. L., Reddy, V. S., Gupta, G. A., Thakur, S., Chaudhary, J., Sharma, B., Verma, A., Tamulevicius, S., Thakur, V. K., Queiroz, A. U. B., Collares-Queiroz, F. P., PRÁ, M. C. DE, Pathak, S., Sneha, C., Mathew, B. B., Muhammad Shamsuddin, I., Karan, H., Funk, C., Grabert, M., Oey, M., Hankamer, B., Kale, G., Kijchavengkul, T., Auras, R., Rubino, M., Selke, S. E., Singh, S. P., Kab, H., Jabeen, N., Majid, I., Nayik, G. A., Huneault, M. A., Li, H., Gironi, F., Piemonte, V. and Gill, M. (2014) *International Journal of Emerging Technology and Advanced Engineering Study of Bio-plastics As Green & Sustainable Alternative to Plastics*, *Cogent Food &*

Agriculture. Cogent, 2(4), pp. 84–90. doi: 10.1016/j.polymer.2006.11.023.

Xu, Z., Niu, Y., Wang, Z., Li, H., Yang, L., Qiu, J. and Wang, H. (2011) *Enhanced nucleation rate of polylactide in composites assisted by surface acid oxidized carbon nanotubes of different aspect ratios*, ACS Applied Materials and Interfaces. American Chemical Society, 3(9), pp. 3744–3753. doi: 10.1021/am200932q.

Yang, W., Fortunati, E., Dominici, F., Giovanale, G., Mazzaglia, A., Balestra, G. M., Kenny, J. M. and Puglia, D. (2016) *Synergic effect of cellulose and lignin nanostructures in PLA based systems for food antibacterial packaging*, European Polymer Journal. Elsevier Ltd, 79, pp. 1–12. doi: 10.1016/j.eurpolymj.2016.04.003.

Yildiz, G., Wehling, R. L. and Cuppett, S. L. (2003) *Comparison of four analytical methods for the determination of peroxide value in oxidized soybean oils*, Journal of the American Oil Chemists' Society, 80(2), pp. 103–107. doi: 10.1007/s11746-003-0659-3.

Zhang, S. M., Liu, J., Zhou, W., Cheng, L. and Guo, X. D. (2005) *Interfacial fabrication and property of hydroxyapatite/polylactide resorbable bone fixation composites*, Current Applied Physics. North-Holland, 5(5), pp. 516–518. doi: 10.1016/j.cap.2005.01.023.

APPENDICES

Appendix A - Calculation of total polyphenolic content in gallic acid equivalent (GAE)

The sample absorbance was measured in triplicate and the absorbance values are as follows;

0.472, 0.464, 0.470.

GAE calculations were made for each absorbance value and presented as the final mean \pm SD.

Representative calculation is as in below;

$$\text{GAE (mg GAE / 1g dw) =} \\ \frac{[(A - 0.0648) * V_{\text{solvent}}(\text{ml})]}{\text{slope of calibration curve} * \text{extract amount (g)}}$$

35.12 mg GAE / 1 g dw was obtained for the absorbance of 0.472.

Where,

GAE: gallic acid equivalent

0,0648: constant from the calibration curve equation (Figure 12)

V_{solvent} : solvent volume to dissolve the ASE

Extract amount: weighted ASE to dissolve

Appendix B - Calculation of transparency

Values of T_{600} of neat PLA and PLA-ASE films were recorded in triplicate for each film sample to calculate the transparency value. Transparency values were calculated for each replicate each of film sample and presented as the final mean \pm SD.

Film thicknesses are 0.153 ± 0.01^a , 0.183 ± 0.02^a , 0.25 ± 0.09^b , 0.27 ± 0.018^b mm for neat PLA, 1.8 wt.% PLA-ASE, 3.2 wt.% PLA-ASE, 4.6 wt.% PLA-ASE, respectively.

Representative calculation for a neat PLA film is as in below;

T_{600} : 53.7 %

$$T = -\log\left(\frac{T_{600}}{X}\right)$$

12.1 was obtained as transparency.

Where,

T: transparency

T_{600} : transmittance at 600 nm

X: film thickness (mm)

Appendix C - Calculation of water vapor permeability

WVP test was performed in triplicate and values of WVP were calculated for each film sample and then presented as the final mean \pm SD.

Film thicknesses are $0.00015 \pm 1.05 \times 10^{-5}$, $0.00018 \pm 2.34 \times 10^{-5}$, $0.00025 \pm 0.9 \times 10^{-5}$, $0.00027 \pm 1.82 \times 10^{-5}$ mm for neat PLA, 1.8 wt.% PLA-ASE, 3.2 wt.% PLA-ASE, 4.6 wt.% PLA-ASE, respectively.

Exposed film areas are 0.00019 ± 0 , $0.00023 \pm 2.9 \times 10^{-5}$, 0.00019 ± 0 , and $0.00021 \pm 2.9 \times 10^{-5}$ m² for neat PLA, 1.8 wt.% PLA-ASE, 3.2 wt.% PLA-ASE, and 4.6 wt.% PLA-ASE, respectively.

The weight changes of jars of the tested film samples were periodically recorded as a function of time and the resulting graphs, which are representative one of three replicates, were shown below.

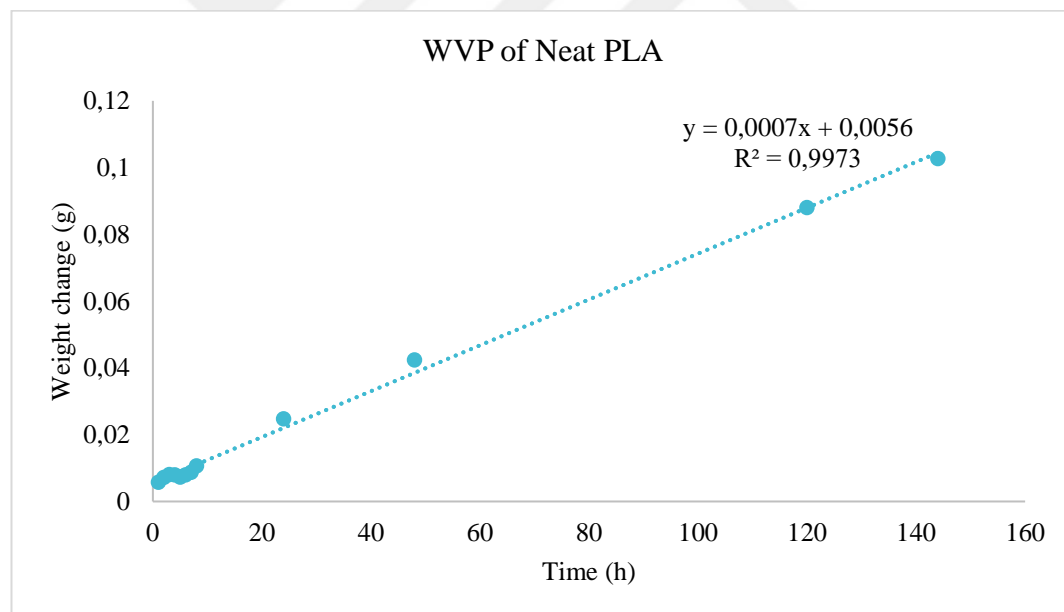


Figure C1. Weight changes of the tested jar against the time for neat PLA.

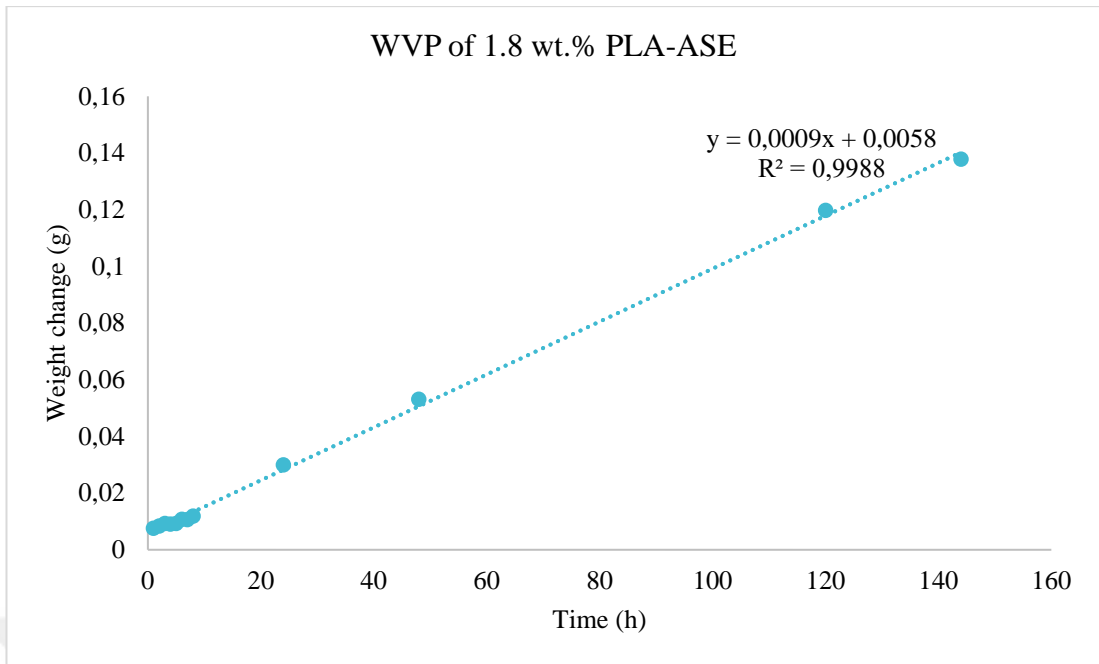


Figure C2. Weight changes of the tested jar against the time for 1.8 wt.% PLA-ASE.

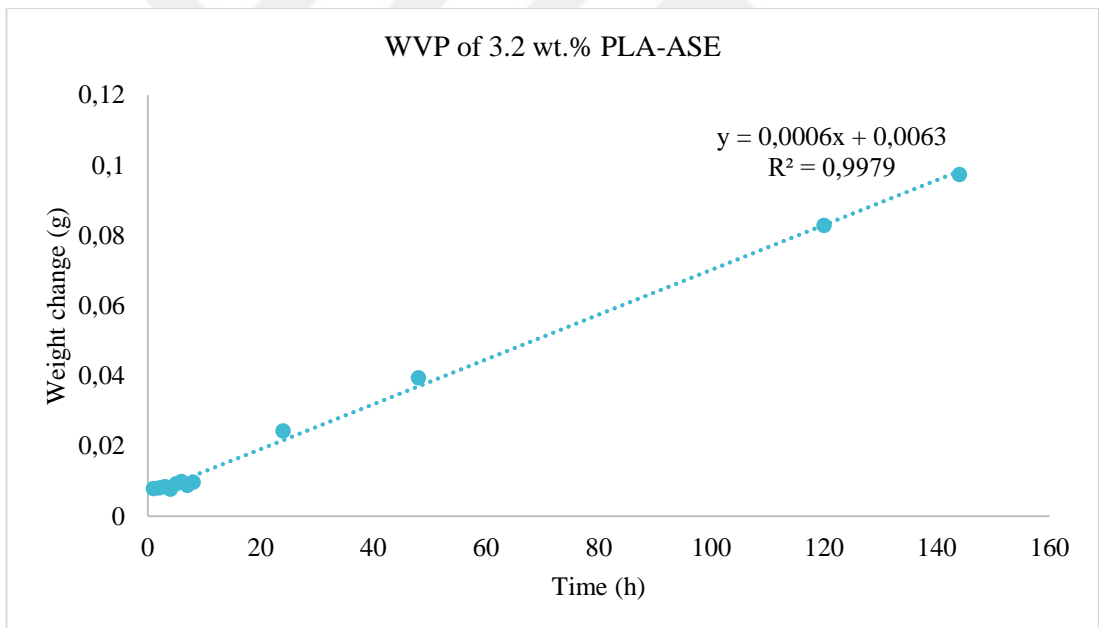


Figure C3. Weight changes of the tested jar against the time for 3.2 wt.% PLA-ASE

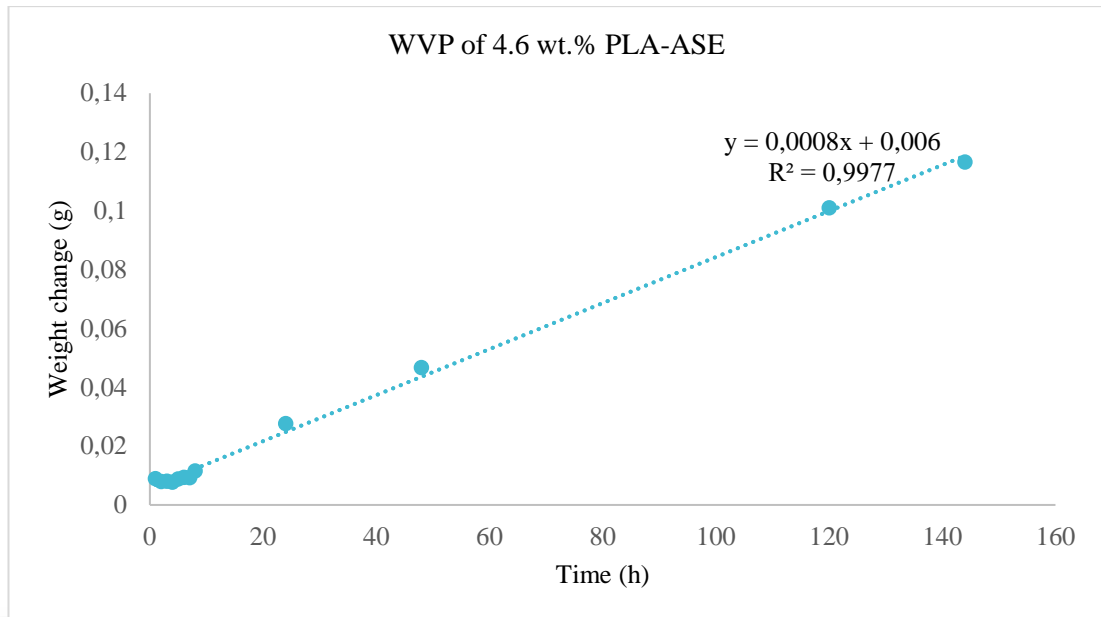


Figure C4. Weight changes of the tested jar against the time for 4.6 wt.% PLA-ASE.

Representative calculation for a 3.2 wt.% PLA-ASE film is as in below;

$$WVP = \frac{\Delta W * x}{t * A * \Delta P}$$

Where,

$\Delta W / t$: slope of the graph

X: average film thickness (m)

A: exposed film area (m²)

ΔP : water vapor pressure difference across the two sides of the film (3169 Pa)

$2,43 \times 10^{-7} \text{ gm}^{-1} \text{ h}^{-1} \text{ Pa}^{-1}$ was obtained as value of WVP.

Appendix D - Calculations for oxygen permeability

To understand the oxygen permeability of the tested film samples, PV value measurement was performed in triplicate for each film sample. The results were presented as the final mean \pm SD in milliequivalents peroxide/1000 g of oil (meq/kg) unit.

Representative calculation for a 1.8 wt.% PLA-ASE film as in below;

Volume of Na₂SO₃ for 1.8 wt.% PLA titration: 15.4 ml

Volume of Na₂SO₃ for blank titration: 0.5 ml

$$\text{Peroxide value } \left(\frac{\text{meq}}{\text{kg}} \right) = \frac{(S - B) \times M \times 1000}{\text{g of test sample}}$$

29.8 PV (meq/kg) was obtained.

Where,

S: ml of Na₂SO₃ for sample titration

B: ml of Na₂SO₃ for blank titration

M molarity of Na₂SO₃ solution

Appendix E - Calculation of radical scavenger activity

DPPH test method was performed in triplicate for each film sample. The results presented as the final mean \pm SD in RSA (%).

Absorbance values of methanolic solution were recorded at 517 nm.

Representative calculation for a 4.6 wt.% PLA-ASE film is as in below;

Abs₅₁₇ of 4.6 wt.% PLA-ASE: 0.9398

$$RSA (\%) = \left[\frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \right] \times 100$$

25.55 % RSA was obtained.

Where,

Abs_{control}: absorbance of the control and

Abs_{sample}: absorbance of the sample

Two separate group anova analyzes were performed for the results. Analysis results are as shown below.

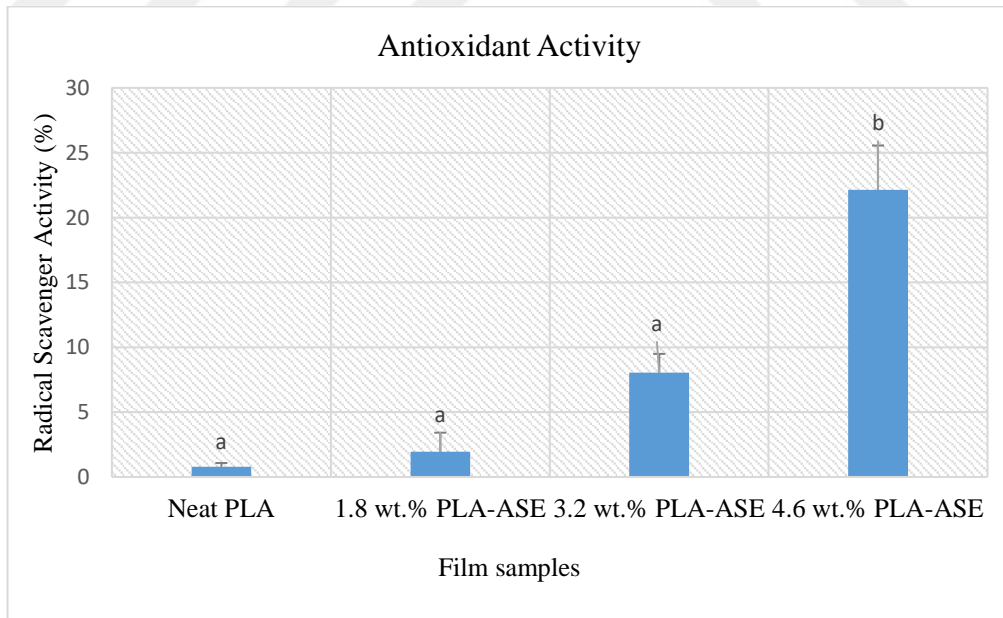


Figure E1. RSA values of neat PLA and three concentrations of PLA-ASE film samples. The different letters on the bars are significantly different ($p < 0.05$). Error bars represent the standard deviations ($n=2$).

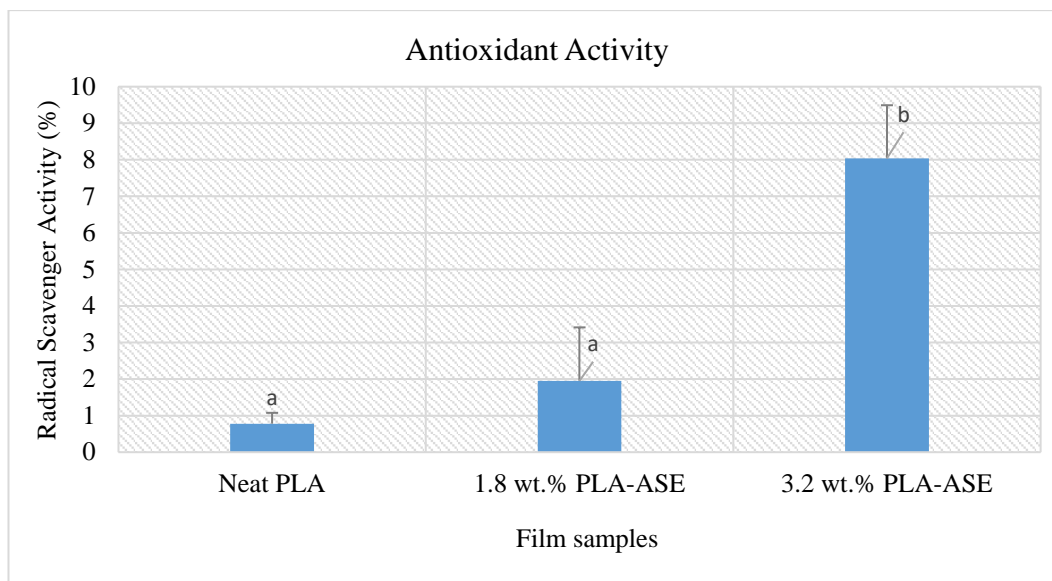


Figure E2. RSA values of neat PLA and two concentrations of PLA-ASE film samples. The different letters on the bars are significantly different ($p < 0.05$). Error bars represent the standard deviations ($n=2$).

In the text, these results were shown in a single chart.

Appendix F - Calculation of crystallinity degree

Thermal transition points of the neat PLA and PLA-ASE film samples were measured with DSC in duplicate. The results presented as the final mean \pm SD.

Representative calculation of X_c (%) for a 1.8 wt.% PLA-ASE film;

$$(\Delta H_m) = 33.02 \text{ J/g}$$

$$(\Delta H_{cc}) = -0.98$$

$$X_c(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_f} \right] \times 100$$

37.85% X_c was obtained.

Where,

ΔH_m : melting enthalpy

ΔH_{cc} : cold crystallization enthalpy

ΔH_f : fusion enthalpy of 100% crystalline PLA which is 93.7 J/g