Citrus Limon L. Peel Powder Incorporated Polyvinyl Alcohol/Corn Starch Antioxidant Active Films

Pınar TERZİOĞLU1*, Yusuf SICAK2

ABSTRACT: Active food packaging films has gain more significance over conventional packaging. In this study, novel active composite films were prepared by incorporating lemon peel to a polyvinyl alcohol-starch matrix. The morphological, thermal and antioxidant activity evaluation of polyvinyl alcohol-starch-lemon peel composite films were discussed to determine whether the presence and content of lemon peel influenced the film’s characteristics. The results indicated that tested properties of composite films depended on the content of lemon peel. It was found that the thermal stability and antioxidant activity of films considerably improved upon the incorporation of peel. The prepared films with enhanced antioxidant activity may be used for preservation of oxidation-sensitive food products. The results showed that lemon by-product has great potential to be evaluated into added-value products for functional packaging applications. The developed packaging films will be new alternatives for food preservation materials.

Keywords: Active Packaging, Agro-waste, Biodegradable Polymer, Lemon Rind, Radical Scavenging

1 Pınar TERZİOĞLU (Orcid ID: 0000-0003-4114-7044), Bursa Technical University, Faculty of Engineering and Natural Sciences, Department of Polymer Materials Engineering, Bursa, Turkey
2 Yusuf SICAK (Orcid ID: 0000-0003-2339-5837), Muğla Sıtkı Koçman University, Köyceğiz Vocational School, Department of Medicinal and Aromatic Plants, Muğla, Turkey

*Corresponding Author: Pınar TERZİOĞLU, e-mail: pinar.terzioglu@btu.edu.tr
INTRODUCTION

Food packaging sector is representing more than half of all packaging applications that has a significant role in the modern economy (Nemes et al., 2020; Ludwicka et al., 2020). The most extensively used food packages are fabricated from petroleum-derived polymers, although they are known to have negative effect on the environment due to being resistant to degradation. Hence, the environmental concerns and growing consumption bring along sustainable packaging requirements (Crizel et al., 2018). Moreover, there has been rising demand from consumers for high quality and minimally processed foods which result in the development of novel packaging concepts (Yu et al., 2019; Apicella et al., 2019). Among the innovative packages (aseptic, microwaveable, modified atmosphere, smart etc.) that emerged in the global market, active packaging systems are gaining a particular interest due to surpassing the traditional food packaging materials with additional functions (Hanani et al., 2019; Belan et al., 2019). In this dynamic technology, it is aimed to maintain food quality generally by the release of bioactive agents from the packaging. Especially, antioxidant including films could be used to prevent oxidative damage in fatty foods (Moreno et al., 2015). In this context, the development of biodegradable packages containing bioactive compounds obtained from waste materials is among the top sustainable active packaging trends.

Polyvinyl alcohol (PVA)-starch composite films were widely investigated green alternatives to produce eco-products for packaging applications. The main advantages of combining these two polymers can be concluded as overcoming the low processability and poor mechanical strength of starch while maintaining the biodegradability and low price of the composites (Popescu et al., 2018; Chen et al., 2020). Various studies have been reported for the preparation of PVA-starch films with improved properties (Junlapong et al., 2019; Chen et al., 2020; Kong et al., 2020). Although PVA-starch composites are having reasonably good features, researches are ongoing to append additional properties including bioactivity. This can be achieved by incorporating organic or inorganic fillers. Recently, the addition of agricultural wastes such as pomegranate peel (Hanani et al., 2019), apple peel (Riaz et al., 2020) and papaya peel (de Moraes Crizel et al., 2018) opened a new path to gain bioactivity to polymer composites for active packaging applications. All studies presented an important enhancement in the antioxidant activity of films demonstrating the films can be evaluated as active packaging materials.

Lemon (*Citrus limon* L.) peel is such a residue eventuated from fruit juice processing (Tomar and Akarca, 2019). The peels compose approximately more than half of the fresh lemon weight (Jagannath et al., 2019; Simeone et al., 2020). A little quantity of lemon peel has been used in the fragrance, food and pharmaceutical industry (El-ghfar et al., 2016; Saleem and Saeed, 2020). However, the rest of the peels generates considerable pollution problems. Since the management of citrus waste is costly, the proper evaluation of them is necessary (Anticona et al., 2020). Lemon peel contains many bioactive compounds as essential oils, phenolic acids, flavonoids (flavanones, flavonols, and flavones), vitamins and minerals as well as carbohydrates, pectin, crude fiber (Papoutsis et al., 2016). These functional ingredients have been linked to diverse biological activities including antioxidant and antimicrobial activities. The use of lemon husk in active packaging appears as an interesting inexpensive alternative.

There is no research on the development of antioxidant active films containing lemon peel. The principal goal of this work was the evaluation of the antioxidant capacity of PVA-starch based active packaging materials containing waste lemon husks. The active composite films were examined by thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). Antioxidant activities of the composite films were also evaluated by using ABTS cation radical scavenging, DPPH radical scavenging, β-carotene-linoleic acid and cupric reducing antioxidant capacity assays.
MATERIALS AND METHODS

Materials
Poly(vinyl alcohol) (87.16% hydrolysis degree, 95.4% purity) was obtained from Zag Kimya (TURKEY). Corn starch and citric acid was purchased from Güneş Company and Aksu Company (TURKEY), respectively. Copper (II) chloride, glycerol, sodium hydroxide and sodium chloride were purchased from Merck (Darmstadt, GERMANY). Butylated hydroxytoluene (BHT), α-tocopherol, β-carotene, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), linoleic acid and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, GERMANY). Lemon peel was purchased from Naturelka (Aydın, TURKEY). The lemon peel sieved using a steel mesh sieve (<200 µm). All other chemicals and solvents were of analytical grade.

Experimental procedure
Preparation of active films
The five different active films were prepared by casting method (Table 1) according to the method of Terzioglu and Parın (2020) with slight modifications. The PVA film solution (8% w v⁻¹) was prepared in distilled water under magnetic stirring (700 rpm) at 90°C. Starch (2% w v⁻¹) was gelatinized (95°C, 15 minutes). Subsequently, gelatinized corn starch mixed with the PVA solution under continuous stirring (70°C, 60 minutes). The homogenous solution was cooled to 50°C. Then citric acid (10%, w w⁻¹ of total polymer weight) was added and stirred for 10 minutes. Then, glycerol (20%, w w⁻¹ of total polymer weight) as a plasticizer and Tween 80 (50 µL) as an emulsifier was added to the mixture under constant stirring for 10 minutes, respectively. Finally, the lemon peel powder at concentrations of 1, 2, 4 and 8% w/w based on total polymer weight was added to the PVA-starch film solution. The film solution (15 mL) was cast onto the Exoglass tart mould (10 cm diameter) and allowed to be dry at 40 °C for 24 hours. PVA-Corn starch without lemon peel was also produced as a control film.

Table 1. The composite films formulation

| Sample Code | PVA (w v⁻¹ %) | Starch (wv⁻¹ %) | Lemon peel (wt % of total polymer) | Glycerol (wt % of total polymer) | Citric acid (wt % of total polymer) |
|-------------|----------------|----------------|----------------------------------|---------------------------------|-----------------------------------|
| L0          | 8              | 2              | 0                                | 20                              | 10                                |
| L1          | 8              | 2              | 1                                | 20                              | 10                                |
| L2          | 8              | 2              | 2                                | 20                              | 10                                |
| L3          | 8              | 2              | 4                                | 20                              | 10                                |
| L4          | 8              | 2              | 8                                | 20                              | 10                                |

Characterization of active films
Thickness of films
The film thickness was measured by a digital caliper (ABS ASİMETO, Turkey). Measurements were randomly taken at three locations on each specimen. The results were expressed as the mean of measurements.

Surface morphology of films
The surface of the films was recorded using a scanning electron microscope (SEM JEOL JSM-7600 F, JAPAN). The films were coated with gold (Emitech K550, ENGLAND) before SEM analysis.
Thermal properties

Thermogravimetric analyses (TGA) of the films were conducted on a TGA STA 6000 analyzer (Perkin Elmer™ Instruments, USA) as described by Parrin et al. (2020).

Determination of antioxidant activity

DPPH free radical scavenging and ABTS cation radical scavenging assays were conducted as described by Ferreira et al. (2014). The procedure defined by Başaran et al. (2019) was used to determine the β-Carotene-linoleic acid and cupric reducing antioxidant capacity (CUPRAC) of films with some adjustments. Solutions of films were prepared at 50, 25, 12.5 and 6.25 ppm concentrations. The used reference compounds were BHT and α-tocopherol. The antioxidant activity results of β-carotene-linoleic acid, DPPH scavenging, and ABTS\(^{+}\) scavenging activity assays are presented as 50% inhibition concentration (IC\(_{50}\)), while CUPRAC capacity activity assay results were given as absorbance (A\(_{0.50}\)). The antioxidant activity assays results were performed on a 96-well microplate reader, SpectraMax 340PC384, Molecular Devices (USA).

RESULTS AND DISCUSSION

Film thickness

Film thickness is a key factor that influences the various properties of films including barrier, optical and mechanical (Marvdashti et al., 2017). The thickness of films varied from 0.22 to 0.26 mm (Table 2). Generally, the films with 4 wt% and 8 wt% lemon peel powder were slightly thicker than the other films. The rise of film thickness with the addition of lemon peel could be related to the increase of solid content in the film forming solution. Our results are in agreement with the studies reported by Moghadam et al. (2020) and Hanani et al. (2019) who examined the effects of pomegranate peel powder addition on the thickness of mung bean protein and fish gelatin films, respectively.

Table 2. Thickness of PVA-Corn starch-Lemon peel composite films

| Sample | Thickness (mm) |
|--------|----------------|
| L0     | 0.22±0.01      |
| L1     | 0.22±0.01      |
| L2     | 0.22±0.01      |
| L3     | 0.23±0.01      |
| L4     | 0.26±0.02      |

The selection of the suitable antioxidant additive to be incorporated in the packaging material has great importance. The antioxidant additive and the packaging material should be compatible to obtain a homogeneous distribution (Gomez-Estaca et al., 2014). The thicknesses of the prepared PVA-Corn starch-Lemon peel composite films were homogeneous indicating the good compatibility of the selected matrix materials.

Microstructures of the lemon peel and films

Scanning electron microscopy micrographs of the lemon peel powders are presented in Figure 1. It is clear that the lemon peel particles have heterogeneous size and irregular shape with rough texture.

Scanning electron micrographs of neat PVA-Corn starch (L0) and PVA-Corn starch-Lemon peel composite films (L1-L4) are shown in Figure 2. The embedded granules of starch in a continuous PVA matrix was observed from the SEM image of neat PVA-Corn starch film. Similar morphology was reported by Das et al. (2010) for glycerol and epichlorohydrin cross-linked starch-PVA films. The incorporation of lemon peel increased the homogeneity of the film surface which could be related to
hydrogen bonding between the -OH groups of lemon peel, PVA and starch (Boonsuk et al., 2020). Additionally, this showed the better adhesion and compatibility between peel and matrix (Singha et al., 2015). The surface of lemon peel incorporated composite films were relatively smooth that have a continuous matrix with no pores and good structural integrity. Very sparsely distributed small particles were observed on the surface of the films. Moghadam et al. (2020) also observed that when pomegranate peel was incorporated into mug-bean protein films, white spots occurred in the SEM micrographs based on availability of insoluble fibers in the peel. Cross-sections of films were also examined with SEM. The composite films exhibited a rough cross-section. Especially, the cross section of L4 sample showed increased heterogeneity with pores and cracks.

![Figure 1. SEM micrographs of lemon peel powder a. x 100 b. x 250 magnification.](image)

**Thermal analysis of the films**

It is very significant to determine the thermal properties and stability of the packaging films because there are large temperature variations according to application such as frozen storage and heat processes during the preparation or processing of foods (Rešček et al., 2018; Picchio et al. 2018).

Thermal decomposition curves, thermal stability and degradation temperatures of the composite films incorporated with lemon peel at different concentrations were presented in Figure 3 and Table 3. All films exhibited similar degradation pattern. The TGA curves imply that the incorporation of lemon peel increased the thermal stability. Especially the film with the highest amount (8 wt %, L4) of lemon peel showed higher resistance against early degradation when compared to the neat film. It was also clear from the 90 wt % loss of L4 and control (L0) sample since the 90 wt % loss temperature of the film was extended from 455 to 474°C with lemon peel incorporation. The presence of lemon peel enhanced the thermal stability due to providing physical bonding and good crosslinking between the components of composite film (Sharmin et al. 2020).

The thermal degradation of composite films occurred in five different stages. The initial stage occurred between 50-160°C is related to the loss of water, residual acetic acid and other volatile contents (e.g. glycerol) in composite films (Hiremani et al., 2020). The second stage 220-280°C associated with the degradation of molecules of polymers by dehydration of the hydroxyl group. This stage was followed by the complete volatilization of the polymer products at 390-440°C (Wu et al., 2019). Through the last of the fourth stage (460-600°C) carbonization of residual organic matter (Sin et al., 2011). The last stage was associated with the burning of the carbonaceous residues which was formed under nitrogen
condition. After 650°C, the curve tended to be smooth. The prepared composite films can be safely used up to 210°C as also reported by Sharmin et al. (2020).

![Surface View](image1)
![Cross Sectional View](image2)

**Figure 2.** SEM micrographs of lemon peel incorporated PVA-starch composite films
Table 3. Degradation temperature of films at residual weight 90 % (Td_{90}), 50 % (Td_{50}), and 10 % (Td_{10}) and the residue at 600°C

| Sample | T_{d10} (°C) | T_{d50} (°C) | T_{d90} (°C) | Residual weight (%) at 600°C |
|--------|--------------|--------------|--------------|-----------------------------|
| L0     | 455.40       | 330.23       | 173.52       | 5.86                        |
| L1     | 460.76       | 331.45       | 171.89       | 6.60                        |
| L2     | 459.61       | 328.88       | 172.17       | 6.59                        |
| L3     | 465.09       | 329.90       | 171.39       | 7.07                        |
| L4     | 474.92       | 331.76       | 185.46       | 7.87                        |

Antioxidant activity of films

Oxidation is the primary non-microbial reason of quality deterioration in terms of shelf life, nutritional value and sensory qualities (appearance of rancidity, off-odors & flavors, color, texture etc.) in various foods (Wrona et al., 2021; Choulitoudi et al., 2020). Therefore, the antioxidant activity of films is a significant active function in the food packaging context due to having a pivotal role in food quality as well as preservation against oxidation (Moreirinha et al., 2020).

The effect of lemon peel on the bioactivity of PVA-starch composite films was evaluated through four different antioxidant activity assays (Table 4). Films showed lower antioxidant activity than lemon peel powder. The results demonstrated that the antioxidant activity of the PVA-starch film was significantly increased with the increase of lemon peel content. The PVA-starch film with 8% lemon peel had the highest radical scavenging capacity. Lemon peel is a well-known rich source of bioactive compounds that are potent natural antioxidants such as essential oil, flavonoids, phenolic compounds and vitamins (Zhang et al., 2018; Wang et al., 2020; Elkhatim et al., 2018). The use of lemon peel containing films might be advantageous for the packaging of foods that have high sensitivity to oxidation.

Similar results were reported in the study of Gaikwad et al. (2016) on the antioxidant potential of apple pomace incorporated PVA active food packaging films. The findings of the study indicated that the incorporation of apple pomace to PVA films enhanced the DPPH scavenging activity of the biocomposite films. It was stated that the antioxidant activities of composite films were lower than the apple pomace as well as the antioxidant potential of the films were increased with the increment of a-pomace amount. Our results were also in agreement with those of Zhang et al. (2019) who reported significant enhancements in the antioxidant ability of composite films composed of chitosan and mangosteen (Garcinia mangostana L.) rind powder. Moreover, Hanani et al. (2019) reported that the addition of an antioxidant biowaste, pomegranate peel powder, to fish gelatin films improved the antioxidant properties of the films in both DPPH and ABTS radical-scavenging activity assays.
Table 4. Antioxidant potential of PVA-starch films containing different amounts of lemon peel powder

| Sample | DPPH scavenging (IC_{50} µg mL⁻¹) | ABTS scavenging (IC_{50} µg mL⁻¹) | β-carotene/linoleic acid assay (IC_{50} µg mL⁻¹) | CUPRAC capacity (A_{0.5} µg mL⁻¹) |
|--------|----------------------------------|----------------------------------|-----------------------------------------------|----------------------------------|
| LO     | 31.16±0.24                      | 40.18±0.34                      | 43.54±0.72                                    | 36.27±0.01                      |
| L1     | 28.71±0.46                      | 32.02±0.86                      | 38.10±0.25                                    | 30.15±0.00                      |
| L2     | 26.09±0.33                      | 25.35±0.69                      | 32.26±0.68                                    | 29.36±0.02                      |
| L3     | 24.24±0.50                      | 22.52±0.37                      | 28.61±0.53                                    | 24.18±0.00                      |
| L4     | 22.07±0.13                      | 20.01±0.73                      | 26.82±0.41                                    | 23.75±0.01                      |
| Lemon peel | 10.06±0.19                  | 19.73±0.08                      | 13.20±0.11                                    | 10.03±0.03                      |
| α-TOCb | 12.26±0.07                      | 4.87±0.45                       | 4.50±0.09                                     | 40.55±0.04                      |
| BHTb   | 54.97±0.99                      | 2.91±0.55                       | 2.34±0.09                                     | 4.00±0.04                       |

a Values expressed are means ± SD of three parallel measurements. p < .05, significantly different with student’s t-test.
b Reference compounds.

CONCLUSION

In this study, a new approach was developed to add value to lemon husk by a cost-effective way to obtain functional packaging materials. Active composite films composed of PVA, starch and lemon peel powder were successfully prepared by solvent casting technique. The morphological analysis showed that the addition of lemon peel increased the homogeneity of the film surface. The thermal stability and bioactivity of films enhanced with the incorporation of lemon peel. The films are potential low-cost antioxidant active packaging candidates with the purpose to broaden the shelf-life foodstuffs.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author’s Contributions

The authors declare that they have contributed equally to the article.

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