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DEVELOPMENT OF BIODEGRADABLE ACTIVE FOOD PACKAGING FROM AGRICULTURAL WASTES: PRODUCTION, CHARACTERIZATION, AND APPLICATION OF FILMS WITH BANANA PEEL AND OLIVE LEAF EXTRACT

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ABSTRACT
The objective of this study is to produce a biodegradable active food package by using agricultural wastes; banana peel and olive leaf extract (OLE). To investigate the possible effect of plasticized starch at different concentrations (0%, 5%, 10%, and 15%), corn starch was added to the films. The films were analyzed in terms of physical characteristics (moisture content, swelling degree, water solubility, density, opacity), water vapor permeability, and mechanical properties. In addition, the phenolic content and antioxidant activity of the films were measured and antimicrobial activity of the films were tested on common food-borne pathogens. Films with better characteristics (B_S15_OLE) were selected to investigate the possible influence of the active films on shelf life of strawberries. Finally, it was shown that at the end of 10days, the films were almost completely decomposed.
Keywords: Agricultural waste, banana peel, olive leaf extract, active film

TARIM ATIKLARINDAN BİYOBOZUNUR AKTİF GIDA AMBALAJI GELİŞTİRİLMESİ: MUZ KABUĞU VE ZEYTİN YAPRAĞI EKSTRESİ İLE FİLMLERİN ÜRETİMİ, KARAKTERİZASYONU VE UYGULAMASI

ÖZ
Bu çalışmanın amacı, tarımsal atıkları; muz kabuğu ve zeytin yaprağı ekstresi (OLE) kullanılarak biyolojik olarak parçalanabilen aktif bir gıda paketi üretilmesidir. Farklı konsantrasyonlarda (0%, 5%, 10% ve 15%) plastikleştirilmiş nişastanın olası etkisini araştırmak için filmlere mısır nişastası eklenmiştir. Filmler fiziksel özellikler (nem içeriği, şişme derecesi, su çözünürlüğü, yoğunluk, opaklık), su buharı geçirgenliği ve mekanik özellikler açısından analiz edilmiştir. Ayrıca, filmlerin fenolik içeriği ve antioksidan aktivitesi ölçülmüş ve filmlerin yaygın gıda kaynaklı patojenlere karşı antimikrobiyel aktivitesi test edilmiştir. Daha iyi özelliklere sahip filmler (B_S15_OLE), aktif filmlerin çileklerin raf

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ömrü üzerindeki olası etkisini araştırmak için seçilmiştir. Son olarak, 10 günün sonunda filmlerin neredeyse tamamen parçalandığı gösterilmiştir.
Anahtar kelimeler: Tarımsal atık, muz kabuğu, zeytin yaprağı ekstresi, aktif film

INTRODUCTION

Processing of biomass for the purpose of converting energy, chemicals and materials is called biorefinery. The main purpose of the biorefinery is to maintain sustainable development with ensuring energy security, minimizing climate change and moderating requirement of chemicals and materials (De Albuquerque vd., 2019).

While producing high value-added products from low-value raw materials, readily available raw materials, low cost and high utility value are the main requirements (Lee vd., 2023).

Banana is one of the most widely cultivated fruits in the world. According to FAOSTAT, its production amount reached approximately 135 million tons in 2022. The remaining by-product of the fruit is the banana peel. The fruit and peel ratio may range between 1.4 to 4.1 depending on the ripening of the banana (Silva vd., 2024). However, the most common use of banana peel is as animal feed. Due to the high amount of water content and organic composition of the banana peel, the landfill elimination of peels is not allowed according to law currently in force (Santiago vd., 2022).

Although the composition of the banana peel changes depending on the ripeness, growing season and species, it is a good source of carbohydrate mainly cellulose, hemicellulose, pectin and lignin (Pereira vd., 2021; Chandrasekar vd., 2023). In the literature, the approximate compositional analysis of banana peel has been reported as follows: starch content 3.5-6.3%, resistant starch 2.3-2.5%, crude fat 2.4-11.6%, crude protein 5.5-7.87%, ash 9-11%, and carbohydrate 59.51-76.58% (Mohd Zaini et al., 2022).

Starch is the most abundant biopolymer in nature and composed of amylose and amylopectin units. Heating of starch with the sufficient water will cause irreversible gelatinization of starch and after gelatinization casted starch paste will dried and promote the formation of newly formed hydrogen bonds between amylose and amylopectin molecules (Cui vd., 2021). Further, banana peel is also rich in terms of phenolic, it contains more than 40 active compounds with four main categories; (i) catecholamines, (ii) flavan-3-ols, (iii) flavonols, (iv) hydroxycinnamic acids. Moreover, the antimicrobial and antioxidant activities of these compounds have already been established (Vu vd., 2018).

The compositional variability of banana peels can pose a challenge to maintaining consistent film characteristics, and the presence of different biopolymers may introduce impurities into the film. However, given the large amount of banana peel waste, combining them with starch could provide useful insights for future applications.

Food packaging is the key element in terms of two main aspects. The first one is to provide sustainable food consumption by reducing the waste and the second one is to relieve packaging waste by more sustainable solutions (Guillard vd., 2018). Traditional plastic packages derived from petroleum-based sources decompose over a hundred years in the soil and cause migration of the micro-nano plastics or toxic compounds into the earth. On the other hand, bioplastics are produced either from the renewable sources (starch, protein, cellulose), as the name implies, or the fossil-based sources, (PBAT, PCL) and they are biodegradable (Harnkarnsujarit vd., 2021).

Similar to the pursuits in the sustainable food packaging materials, incorporation of the active agents from the agricultural waste has started gaining attention. Inevitable by-product of the olive oil industry, olive leaf, accounts for millions of tons of waste annually such that only in Spain, 1.25 million tons of leaf have been discarded which corresponds to approximately half of the overall production in the world. Further, antimicrobial, antioxidant, anti-inflammatory and anticancer effects have already been studied (Espeso vd., 2021).

The objective of this study is to test the possibility of production of biodegradable, edible food packages from banana peel. Since banana peel is a source of multi biopolymers, to achieve the desired integrity, modify the film structure and analyze the possible effect, corn starch with various concentrations were incorporated into the banana peel film. Although banana peel includes many phenolic compounds, to improve the active film property of the films, olive leaf extract was added to the film formulation. Solubility, water permeability, mechanical properties, antioxidant activity, and total phenolic content of the films were investigated. The FTIR analysis has been carried out to examine the possible interactions and chemical bonds between banana peel, cornstarch and olive leaf extract. Further, antimicrobial activity of the films was also tested on most common food borne pathogens, *Escherichia coli* and *Staphylococcus Aureus.*

MATERIALS AND METHODS

Materials

Bananas and corn starch (As Gıda Üretim ve Pazarlama A.Ş., Kocaeli, Türkiye) were purchased from a local market in Ankara. Analytical grade hydrochloric acid solution (32–36%) and sodium hydroxide pellets, glycerol (99.5% v/v), DDPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid (MW= 170.12 g/mol), Folin–Ciocalteu reagent, sodium carbonate and ethanol were purchased from Merck (Darmstadt, Germany). Nutrient broth and Mueller–Hinton Agar were obtained from Condalab (Madrid, Spain). Finally, olive leaf extract was bought from Ersağ Bitkisel Sağlık Gıda Ltd Sti (Denizli, Türkiye).

Methods

Film Preparation

The banana peel and corn starch-based films were prepared with a method described by Verma vd., (2024). Banana peels (400g) were cut into small pieces with the stainless knife then immediately soaked into the acetic acid solution (2%, 500 mL) and boiled for 30 mins. To drain the excess water, it was kept at room temperature for 1h. Then, it is transferred into a new beaker with 500 mL water and boiled for 30 min. The boiled suspension was blended (Tefal MasterBlend Activflow Pro 1000 W) for 5 min. In another beaker, corn starch solutions with 5%, 10% and 15% concentrations were prepared. Heated solutions at 85°C were kept at that temperature for 30 min at the magnetic stirrer (MaxTir 500, Daihan Scientific, Seoul, Korea), at 250 rpm. Finally, banana peel (24 mL) and cornstarch (3 mL), glycerol (2mL) were mixed at 1500 rpm in a magnetic stirrer for 15min. Finally, the film forming solutions were transferred into the petri plates and dried at 25°C, at 50% RH for 48h. To produce the olive leaf extract (OLE) added samples, OLE was incorporated into each film forming a solution 10% based on the film's solid content. Films were conditioned in the climate chamber (52%, 20°C) before analysis. The formulation and the nomenclature of the films were given in Table 1. In that table, B and S referred to banana peel and corn starch, respectively. Further, (-) and (+) signs represented absence and presence of OLE, respectively.

Table1: Nomenclature of the films

Nomenclature	Starch solution concentration	OLE
B_S0	0%	-
B_S5	5%	-
B_S10	10%	-
B_S15	10%	-
B_S0_OLE	0%	+
B_S5_OLE	5%	+
B_S10_OLE	10%	+
B_S15_OLE	15%	+

Moisture content, swelling degree, solubility and opacity of the films

Films were cut in to a square $(2cm \times 2cm)$ shape and the first weight of the samples (W_1) were measured. In that step, the thickness (χ) of the films was also measured. Then, they were placed in a hot oven (105°C) and kept until they reached a constant weight (W_2). After this step, dried films were immersed into the 25 mL of water at room temperature (25°C) overnight. After excess water was removed with a soft tissue, the third weight of the samples (W_3) were measured. Finally, the films were put again into the hot air oven for 24h to dry out the samples, at the end of the period, the last weight of the samples (W_4) were measured. Moisture content, swelling degree, solubility, opacity of the films was calculated using the following equation (Yildiz vd., 2022).

Moisture content (MC) (%) = $\frac{W_1-W_2}{W_1} \times 100$ (Equation 1)

Swelling degree (SD) (%) = $\frac{W_3-W_2}{W_3} \times 100$ (Equation 2)

Solubility (%) = $\frac{W_2-W_4}{W_4} \times 100$ (Equation 3)

Opacity= $\frac{A_{600}}{\chi}$ (Equation 4)

Density= $\frac{W_1}{\chi \times 4cm^2}$ (Equation 5)

In equation 4, A_{600} referred to the absorbance of the films at 600 nm, which was measured in a spectrophotometer (Optizen Pop Nano Bio, Mecasys Co., Ltd., Daejeon, Korea).

Water vapor permeability of the films

The water vapor permeability (WVP) of the films were measured by ASTM E96 with some modifications. Cylindrical test cups with the inner diameter of 40 mm are filled with 35 mL of distilled water. Then, the thickness of the films was measured and the films were fixed between the cup and cap to ensure that there was no leakage. The initial weight of the cups was reported and they were transferred in to the desiccator with 10∓2% relative humidity. The test temperature was kept at 25°C. The weight of the cups was reported in 2 h time intervals. Finally, the WVP of the films were calculated by using the following equation.

WVP=$\frac{WVTR \times \Delta x}{S \times (R_1-R_2)}$ (Equation 6)

In the given equation, WVTR represented the water vapor transmission rate and it was the slope the weight loss versus time data. $\Delta x, S, R_1$ and R_2 referred to the thickness of the film, saturated water vapor pressure inside the cup (100%) and inside the desiccator at a given temperature, respectively.

Mechanical properties of the films

The tensile strength (TS, MPa) and elongation at break (EAB, %) values of the films were determined by the texture analyzer (Brookfield, Ametek CT3, Middleboro, MA, USA, TA- DGA tension probe) (Emir vd., 2023). The films were cut into the rectangular shape (2cm× 8cm) and their thickness were reported. The test was performed with a 0.4 N load cell at a test speed of 0.40 mm/s.

TS (MPa) = $\frac{F_{max}}{A}$ (Equation 7)

EAB (%) = $\frac{L}{L_0} \times 100$ (Equation 8)

In given equations, F_{max} is the maximum load (N) film could stand and A is the cross-sectional area (mm^2) which is perpendicular to the applied force. Finally, L_0 and L are the initial and final lengths of the film, respectively.

Total phenolic content (TPC) and antioxidant activity of the films

TPC of the films were measured by Folin-Ciocalteu method with slight modifications (Yildiz vd., 2021). A piece of film (~100mg) was dissolved in ethanol/water mixture (80:20 v/v). Then, it was vortexed for 2 mins and centrifuged (MIKRO 220R Hettich Zentrifugen, Tuttlingen, Germany) at 1500 rpm for 15 min. After this step, 500μL of the supernatant was mixed with 2.5 mL of 0.2N Folin-Ciocalteu reagent. The mixture was vortexed and kept in the dark for 5 min. After introducing 2 mL of 7.5% w/v sodium carbonate into the mixture, it was kept at dark for 1h. After this period of time, the absorbance of the samples was measured by the spectrophotometer at 760nm. The calibration curve of the samples was prepared by gallic acid. Different concentrations of the gallic acid (10, 20, 40, 80 and 100 mg/L) was prepared into ethanol/water mixture (80:20 v/v). The steps mentioned above were repeated for the gallic acid solutions. TPC of the samples were calculated using the equation below.

$$TPC=\frac{C\times V\times D}{W_s}$$ (Equation 9)

In this equation, C value referred to the concentration corresponding to the absorbance value from the calibration curve (mg/L), V, D and Ws were the volume of solution in L, dilution rate and sample weight (mg), respectively.

Antioxidant activity of the samples were obtained by DPPH assay (Aydogdu vd., 2019). DPPH was dissolved in methanol and a 50 ppm DPPH solution was prepared. Then, diluted samples (0.1μL), which was explained in the previous part, were introduced into the prepared DPPH solution (3.9mL). The mixtures were kept in dark at 1h and the absorbance (A_2) of the mixtures were measured by a spectrophotometer at 517 nm. Methanol used as a blank and 0.1mL of methanol solution was mixed with 3.9mL of DDPH solution similar to the sample, and its absorbance (A_1) value was also read at the end of the 1h. Using the DPPH calibration curve, C_1 and C_2 concentration corresponding to the A_1 and A_2 absorbances was calculated. Finally, antioxidant activity of the films was calculated using the equation below.

$$\text{AA (mg DPPH/g of sample)} \frac{C_1-C_2}{W_{sample}}\times V$$ (Equation 10)

In this equation, W_{sample} and V were the weight of sample in g and volume of sample in L, respectively.

FTIR Analysis

FTIR analysis of the films was performed using an FTIR spectrophotometer (IR-Affinity1, Shimadzu, Kyoto, Japan) in attenuated total reflectance (ATR) mode with a diamond ATR crystal. The spectra were recorded with 32 scans over a wavenumber range of 600–4000 cm^{-1} (Aydogdu vd., 2019).

Antimicrobial activity of the films

To promote microbial development, 10 mL of broth were used to inoculate Gram-positive *Staphylococcus aureus* (ATCC 43300) and Gram-negative *Escherichia coli* (ATCC 11229) cultures. The cultures were then incubated at 37 °C for 24 hours. Using a spectrophotometer (UV 2450, Shimadzu, Columbia, USA), the bacterial culture was diluted and its absorbance was adjusted to 0.9 at 600 nm in order to get an inoculum concentration of around 10^8 CFU mL^{-1}. Cut into discs with a diameter of 1 cm, the films were then placed on agar plates that had been previously inoculated with 0.1 mL of the bacterial cultures. After that, the plates were incubated for 24 hours at 37 °C (Emir vd., 2023).

Preservation of strawberries

A batch of strawberries was purchased from a local market in Ankara. Strawberries with similar maturity, size and color were selected and they were free from any physical damages. Strawberries were placed into the active films and they were folded to cover all surfaces of the strawberries. Samples were kept in the refrigerator at 4°C and their photo was taken for 6 days. Strawberries were evaluated according to their visual appearance.

Statistical analysis

To determine if there was a significant difference among the samples, an analysis of variance (ANOVA) was conducted using MINITAB (Version 16). If a significant difference was found, a Tukey multiple comparison test was used for comparison ($P \leq 0.05$). Correlations between the results were calculated using Pearson correlation with a 95% confidence level ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Physical Properties of the Films

Moisture content (MC) is a parameter that shows how much moisture is entrapped into the film matrix and therefore it influences other film characteristics like mechanical and barrier properties (Aydogdu Emir vd., 2023). As seen Table 2, MC of the films ranged from 8.43±0.20% to 18.97±0.23%. These values were lower than sweet lime peel incorporated polyvinyl alcohol/starch-based biodegradable films (Singha vd., 2023) and starch/carboxymethyl cellulose added banana peel films (Verma vd., 2024). MC of the films in the mentioned studies were between 48.7 ± 0.21% - 31.74 ± 0.08% and 31.807±1.280%- 33.241±1.158%, respectively.

Comparing the literature results, starch-banana peel films had a lower or similar moisture content compared to chitosan (27.03±1.84%-17.92±1.30%) and faba bean flour (25.76 ± 1.28 % - 15.11 ± 0.26%) active films.

Table 2: Physical properties of the films with different starch and OLE concentrations

Sample	MC (%)	SD (%)	WS (%)	Opacity	Density (g/cm^3)
B_S0	18.97±0.23[a]	124.59±6.73[b]	44.06±2.33[a]	8.77±0.04[a]	0.86±0.018[b]
B_S5	12.81±0.85[c]	140.09±6.89[ab]	40.50±1.16[ab]	5.34±0.26[cd]	0.86±0.031[b]
B_S10	10.73±0.85[de]	143.13±22.78[ab]	35.82±1.35[bcd]	5.64±0.02[bc]	0.95±0.046[bc]
B_S15	9.33±0.16[ef]	161.71±5.32[a]	28.81±0.99[e]	4.21±0.03[d]	1.25±0.01[d]
B_S0_OLE	16.70±0.35[b]	118.71±5.88[b]	39.01±0.40[bc]	9.05±0.68[a]	0.74±0.01[a]
B_S5_OLE	11.27±0.27[cd]	127.68±0.33[ab]	33.45±0.85[de]	6.76±0.07[b]	0.82±0.06[ab]
B_S10_OLE	8.43±0.20[f]	132.70±0.31[ab]	34.48±0.41[cd]	6.07±0.38[bc]	0.88±0.01[b]
B_S15_OLE	8.44±0.100[f]	138.10±5.18[ab]	19.56±0.72[f]	5.02±0.03[cd]	1.075±0.01[cd]

Different letters in the same column show the significant difference between samples by Tukey's test ($p \leq 0.05$)

According to two-way ANOVA results, incorporation of starch significantly affected the moisture content. Although starch is a highly hydrophilic compound, addition of 5% starch decreased MC and further increasing starch concentration to 10% and 15% caused further reduction in MC (Supplementary file). However, changing starch concentration from 10% to 15% did not have an effect on the MC. Besides, the presence of an active agent (OLE) also had a role in MC of the films. OLE decreased MC of the films significantly (Supplementary file). Similar results were also observed in olive leaf and laurel leaf extract incorporated alginate films. This consequence was explained by the hydrophobic characteristics of the phenolics even though it was in low concentration (Moura-Alves vd., 2023).

According to two-way ANOVA results, incorporation of starch at increasing concentrations led to gradual increasing of SD. B_S15 samples had the highest SD (161.71±5.32%) whereas B_S0 exhibited the lowest value (124.59±6.73%). The main causes of high SD of the films were mainly attributed to the plasticizer (glycerol) and highly hydrophilic nature of the starch due to their free hydroxyl groups. Since glycerol amounts are the same for all films, the primary reason for the increasing SD trend was due to the increasing starch concentration. Similar outcomes were also observed in the study of corn starch-based films (Nasir & Othman, 2021).

To investigate the effect of OLE on SD for all samples, two-way ANOVA analysis was carried out and the results showed that, regardless of the starch concentration, the incorporation of OLE resulted in significant reduction of SD. This reduction might be possible interaction between OLE-starch and components of banana peel. OLE might increase the cohesive intermolecular network and support the formation of strong hydrogen bonds between the film forming components (Yilmaz vd., 2022).

Water solubility of the films is an important phenomenon for the biodegradable and biopolymer-based films. To be biodegradable, films should be loosening their structure when interacting with water. On the other hand, they should keep their integrity when they are utilized in packaging high moist foods (Singha vd., 2023). As mentioned previously, MC has an impact upon the other film characteristics like solubility. The correlation coefficient between MC and solubility was calculated as 0.76 (Pearson correlation with Confidence Interval 95%). Therefore, there was a high correlation between these two parameters. Both starch and OLE had a similar influence on solubility. Both increasing starch concentration and the presence of OLE reduced the solubility of the films (Supplementary file). Compared to films with similar food matrices from the literature, banana peel-starch – OLE films had an acceptable solubility. For example, the solubility of sweet lime peel incorporated polyvinyl alcohol/starch-based biodegradable films (Singha vd., 2023) ranged between 85.09 ± 0.2% and 98.63 ± 0.25% whereas that of banana peel-starch–OLE films were between 44.06±2.33% and 19.56±0.72%. Furthermore, *Eriobotrya japonica* leaves incorporated banana peel starch films' solubility were reported as 34±2% and 42±4% (Medeiros Silva vd., 2020).

Opacity value of the films might change the food type that will be packaged. Lower opacity values might be preferable in packaging if consumers want to see food material in the package (Emir vd., 2024). On the other hand, films with high opacity value are more feasible if food inside the package is more susceptible to UV-light induced changes like lipid oxidation (Emir vd., 2023). The opacities of the films ranged from 4.21±0.03 to 9.05±0.68. Increasing starch concentration caused gradual reduction in opacity values. The reason might be the formation of a denser film structure. Density results, as seen in Table 2, also support this hypothesis. Correlation coefficient between opacity and density was -0.76 (Pearson correlation with Confidence Interval 95%). Therefore, higher starch addition caused more dense structure and increased density and reduced the opacity value.

Further, the surface roughness of the films might also influence the opacity values. Addition of starch might decrease the surface roughness and decrease the opacity as mentioned in the other article (Medeiros Silva vd., 2020).

OLE addition to the samples caused stepwise increase in opacity. The reason for the result might be attributed to the yellowish/brownish color of the OLE extract. In that way, light might be scattered and reflected easily (Emir vd., 2023).

Mechanical Properties of the Films

Tensile strength (TS) can be defined as the maximum amount of load that the film can withstand without fracture. On the other hand, elongation is related to the film flexibility and shows how far material can starch before breaking (Singha vd., 2023). The mechanical properties of the films, TS and EAB were shown in Figure 1. TS of the films was measured between 3.34±0.04 and 9.51±0.01 MPa and these values belonged to the BS_0 and B_15_OLE, respectively. For the films without OLE, increasing starch concentration to 5%, did not significantly change TS compared to B_S0 whereas further increasing starch concentration to 10% resulted in increasing TS by approximately 65%. Furthermore, to investigate the effect of OLE on TS of the films, two-way ANOVA was carried out. The results revealed that OLE added samples had higher TS compared to the control samples (samples without OLE) (Supplementary file) . Therefore, both starch concentration and OLE amount had an impact on TS of the films. Incorporation of starch improved the film integrity which was discussed previously and the results were supported by the density values. Similarly, the possible formation of more cohesive structure due to OLE was also mentioned in the previous part. Banana peel contains many polymers like starch, lignin, pectin, insoluble fibers etc., therefore, these multi component biopolymers might act as impurities and locate the stress at one point and prevent distribution to all film matrices. However, incorporation of starch and OLE contributed to the film forming solution integrity.

B_S5 and BS_15 had the lowest (3.91±0.16%) and the highest (10.44±1.24%) EAB values, respectively. For the control groups (films without OLE), increasing starch concentration increased EAB of the films. Similar outcomes were also reported in another study (Verma vd., 2024). For the films except B_S15 and B_S15_OLE, OLE addition increased EAB values. This result implied that OLE might also behave like a plasticizing agent and made the films more flexible.

Water Vapor Permeability of the Films

The presence of hydrophilic substances like fibers, carbohydrates, and protein and their interactions resulted in more space developing between hydrogen bonds and molecules, which enhances the passage of water vapor through the films and increases the permeability (Verma vd., 2024).

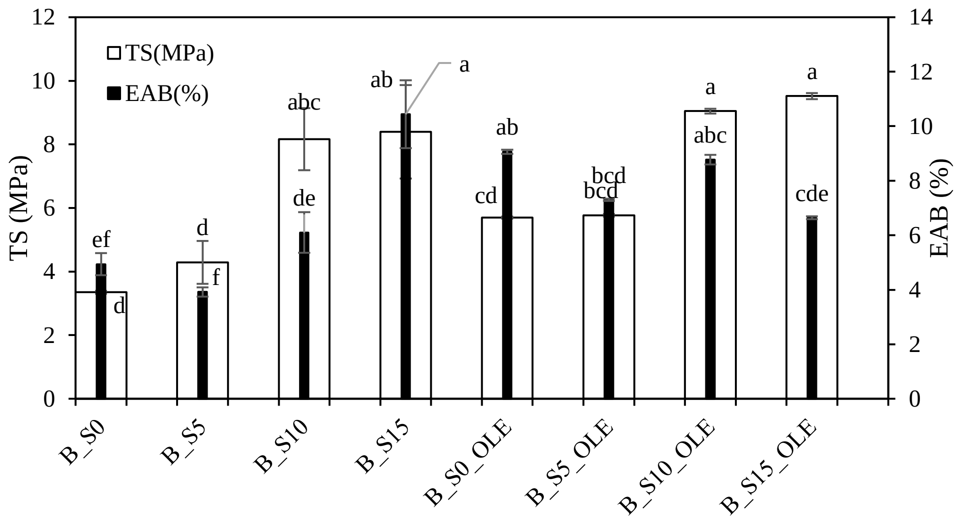

Figure 1: Mechanical properties of the films

WVP of the films was shown in Table 3. As seen, regardless of presence of OLE, 15% starch added samples (B_S15 and B_S15_OLE) had the lowest WVP. Although the common outcomes from the literature supported the idea that increasing hydrophilic compound amount in the film resulted in the increasing permeability. This expectation was caused by the idea that fibers, starch, and proteins could readily interact with the water molecules via hydrogen bonding, in that way, transferring of the vapor molecules form one side to another could be completed easily (Verma vd., 2024). However, this pattern was not observed in banana peel/starch/OLE films. Packing of the film forming components with strong intermolecular and intramolecular hydrogen bonding might obstruct the passing of the vapor molecules. This compact packaging of the film forming components was already supported by the density values of the films (Table 2). The correlation coefficient between density and WVP was found as -0.82 (Pearson correlation with Confidence Interval 95%). Therefore, it could be concluded that increasing density resulted in decreasing permeability values. In addition to that, solubility, which could be interpreted as interaction between film forming components and water showed a parallel pattern with the permeability with the correlation coefficient 0.81. Therefore, films having the high solubility values had a tendency to show high permeability. Finally, it could be concluded that not only film forming components but also physical properties of the films had an influence on WVP.

Presence of active agent (OLE) did not have an impact on the permeability values of the films. Therefore, OLE did not change the hydrophilic/hydrophobic characteristic of the films.

FTIR

FTIR spectra of the sample with and without OLE was illustrated in Figure 2. All films exhibited a peak between 3500-3000 cm^{-1} and this was attributed to the -OH stretching of the hydrogen bonds of water and other films forming components like phenolics, proteins, starch (Medeiros Silva vd., 2020).

Peaks located around 2900 cm^{-1} were due to the C-H stretching vibrations and peaks around 1600-1700 cm^{-1} were attributed to the C=0 stretching of carboxyl groups (Amaregouda vd., 2022). Peaks from 800 cm^{-1} to 1200 cm^{-1} were attributed to C-O-C stretching of all saccharide molecules in the film (Ezati vd., 2022; Bigi vd., 2021) One thing should be noted that films with OLE and without OLE had very similar FTIR spectra, which could be due to the banana peel also contained phenolic compounds and addition of OLE did not modify any different intermolecular interaction chemically.

Table 3: WVP, TPC and AA of the films

	WVP 10^{-9} (g/s.m.Pa)	TPC (mg GAE/g film)	AA (mg DPPH/ g sample)
B_S0	1.76 ± 0.06^a	-	-
B_S5	1.81 ± 0.01^a	-	-
B_S10	1.76 ± 0.01^a	-	-
B_S15	1.61 ± 0.01^b	-	-
B_S0_OLE	1.77 ± 0.02^a	7.150 ± 0.24^c	2.96 ± 0.20^a
B_S5_OLE	1.80 ± 0.01^a	6.56 ± 0.60^{bc}	$2.81\pm0,18^a$
B_S10_OLE	1.74 ± 0.01^a	8.75 ± 0.60^{ab}	3.33 ± 0.27^a
B_S15_OLE	1.59 ± 0.01^b	9.07 ± 0.03^a	3.40 ± 0.12^a

Different letters in the same column show the significant difference between samples by Tukey's test ($p \leq 0.05$)

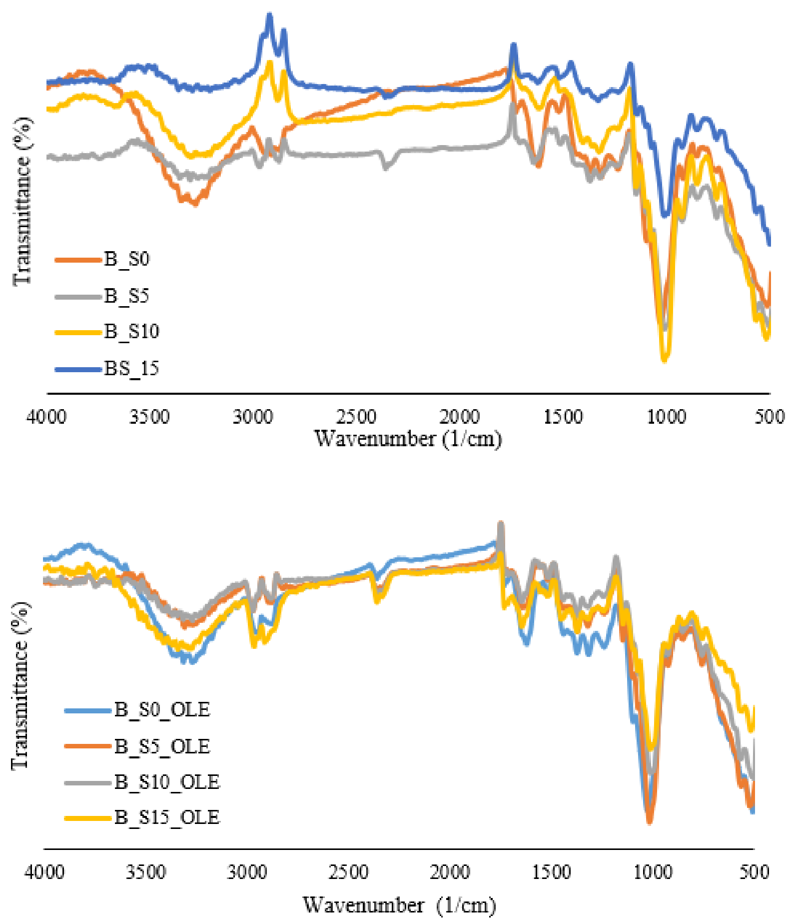

Figure 2: FTIR spectra of the films

Total Phenolic Content, Antioxidant Activity, Film Application on Shelf life of Strawberry & Degradation

TPC and AA of the OLE incorporated films were shown in Table 3. As explained in the method section, OLE was added to the films based on 10% of the solids amount. In correlation with the total solid content, and OLE amount, TPC of the films showed an increasing trend. As a result, B_S15_OLE films had the highest TPC with 9.07±0.03 (mg GAE/g film). It has been identified that banana peel contained more than 40 phenolics and they can be categorized in to four main groups namely; hydroxycinnamic acids, flavonols, flavan-3-ols, catecholamines (Vu vd., 2018) Besides, oleuropein, quercetin, apigenin-7-O-glucoside, luteolin-7-O-glucoside, and verbascoside were the major phenolic groups found in the OLE (Lama-Muñoz vd., 2020). Therefore, not only OLE but also banana peel contributed to the phenolic content of the films. TPC of the OLE-banana peel- starch films were similar to the chitosan-hydroxypropyl methylcellulose (HPMC) films made by nettle or sage leaf extract. TPC of the chitosan-HPMC films were reported between 3.69-9.91 mg GAE/g film (Bigi vd., 2021).

Table 3 showed AA of the films and it ranged between 2.81±0.18 and 3.40±0.12 mg DPPH/ g sample. As known, banana peel contained many saccharides including lignin. The antioxidant activity of the lignin comes from its numerous phenolic units (Lu vd., 2022). Therefore, phenolic compounds, lignin in the banana peel and OLE contributed to overall AA. Although TPC of the films exhibited an increasing trend with an increasing solid amount, AA of all the films were statistically the same. It might be interpreted that incorporation of OLE slightly influenced AA but the natural constituents in the banana peel dominated AA of the films.

The antimicrobial activity of the films were tested on *E.coli* and *S.aureus* and the results were shown in Figure 3. Although films had a significant phenolic content and antioxidant activity, they did not show any antimicrobial activity. This consequence might be explained by the fact that the active inhibitory components in the films were not sufficient to meet minimum inhibitory concentrations (MIC) for the microorganisms. Similar outcomes were also observed in banana peel/agar films; they did not show any inhibitory effect on *L. monocytogenes* and *E. coli* (Orsuwan vd., 2016).

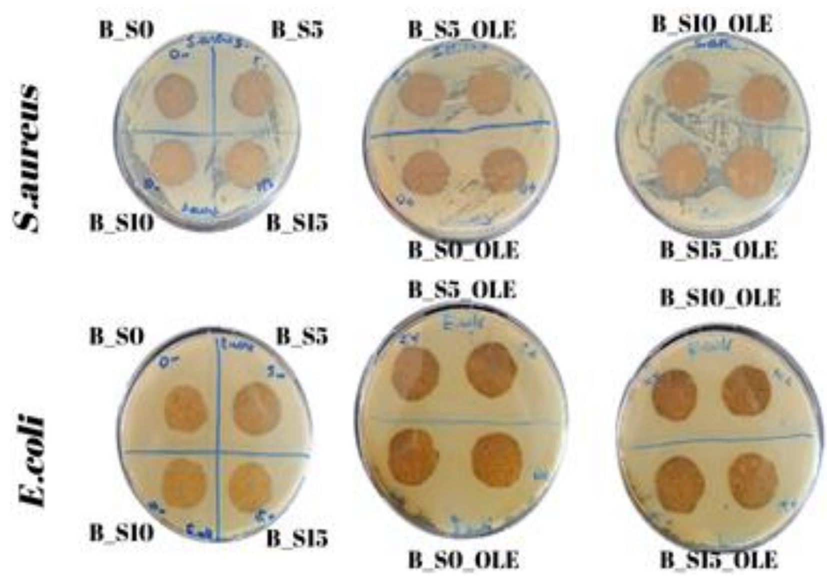

Figure 3: Antimicrobial activities of the films on *S. aureus* and *E. coli*

On the other hand, antimicrobial activity of ethanol extract of banana peel was reported, and the effect was tested on many microorganisms, namely *L. monocytogenes*, *S. aureus*, *E. coli*, *P. aeruginosa* and *S. enteritidis*. Further, MIC of banana peel extract for *P. aeruginosa* was stated as 150 µg/mL (Chandrasekar vd., 2023). MIC of each microorganism is different, however, such a high amount of concentration for the mentioned microorganism supported the idea for why films failed.

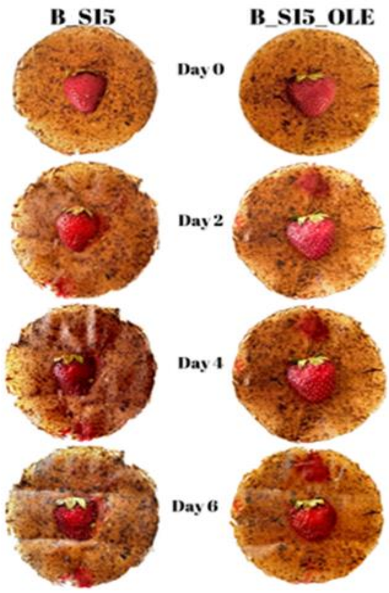

Figure 4: Packaging of strawberries with the banana-starch films for 6 days.

Although film did not exhibit an antimicrobial activity against the selected microorganisms, one of the perishable fruit, strawberry, was chosen to see the possible influence of banana peel/starch /OLE films. For the application, B_S15_OLE film was selected by taking into the consideration of physical properties, WVP, mechanical properties and TPC values. As a control, B_S15 film was chosen. The images of the packed strawberries were shown in Figure 4. While initially both fruits were fresh, on day 4 the fruit packaged with B_S15 began to lose its physical integrity and some damages like exudate were observed. On day-6, although fruit packed with B_S15_OLE had some defects, fruits covered with B_S15 have already decayed. Even if B_S15_OLE films had no inhibitory effect on *S. aureus* and *E. coli*, B_S15_OLE might reduce the total number of microorganisms and therefore retarded the spoilage of strawberry.

Figure 5 shows the soil degradation test of the films over a 10-day period. The films were placed into the torf soil below the 5 cm surface. It was regularly sprayed with water every two days. The pots (10 cm×5 cm) kept under ambient conditions (20°C, 40%RH) (Medina-Jaramillo et al., 2017).

All films lost their integrity by the end of the test period. The results proved that banana peel-starch films, both with and without OLE, were decomposable. The addition of OLE neither sped up nor decreased the decomposition rate. These findings were very similar to the outcomes of the yerba mate extract added to cassava starch films, which also decomposed almost completely at the end of day 12 (Medina Jaramillo vd., 2016).

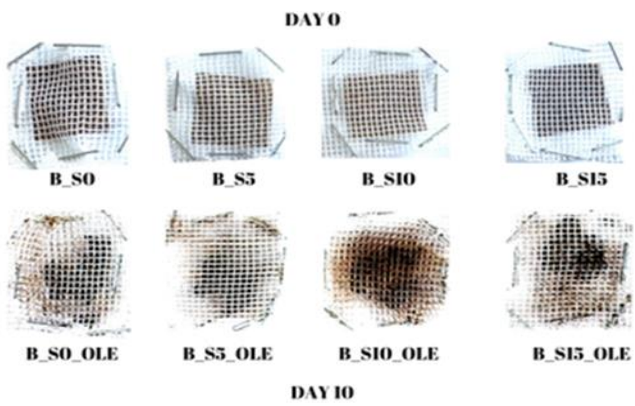

Figure 5: Soil degradation of films through 10-day period

CONCLUSION

Banana peel films with different starch concentration with and without OLE were successfully produced. WS of the films ranged between 44.06±2.33 and 19.56±0.72 %. Increasing starch concentration to 10%, TS increased by 65% compared to the samples without OLE. Further, OLE also enhanced TS of the films. Although films with higher density had a tendency to show low WVP, films with low solubility had higher WVP. Both OLE and the phenolics in the banana peel contributed to TPC. B_S15_OLE films helped to keep fruit integrity for 6 days compared to B_S15.

Considering the limitations, such as water vapor permeability (WVP) and water-soluble characteristics regarding practical applications, other methods, such as crosslinking strategies, may be explored in future studies. Additionally, increasing the amount of OLE in the film structure could help observe the antimicrobial activity more clearly.

CONFLICT OF INTEREST

None

AUTHORS CONTRIBUTIONS

Eda Yildiz: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration; Nur Kus: Conceptualization, Investigation, Resources, Visualization, Methodology; Selen Guner San: Conceptualization, Writing - Review & Editing; Gulum Sumnu: Resources, Writing - Review & Editing

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Supplementary File

Two-way ANOVA results

Mechanical properties

Tensile strength

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
15	4	8.95	A
10	4	8.60	A
5	4	5.02	B
0	4	4.52	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

ole	N	Mean	Grouping
yes	8	7.50	A
no	8	6.04	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch*OLE	N	Mean	Grouping
15 yes	2	9.51	A
10 yes	2	9.04	A
15 no	2	8.39	A B
10 no	2	8.16	A B C
5 yes	2	5.77	B C D
0 yes	2	5.69	C D
5 no	2	4.28	D
0 no	2	3.34	D

Means that do not share a letter are significantly different.

Elongation at Break

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
15	4	8.54	A
10	4	7.43	A B
0	4	6.99	B
5	4	5.60	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

OLE	N	Mean	Grouping
yes	8	7.94	A
no	8	6.35	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch*OLE	N	Mean	Grouping
15 no	2	10.44	A
0 yes	2	9.06	A B
10 yes	2	8.77	A B C
5 yes	2	7.29	B C D
15 yes	2	6.63	C D E
10 no	2	6.10	D E
0 no	2	4.93	E F
5 no	2	3.91	F

Means that do not share a letter are significantly different.

WVP

Grouping Information Using the Tukey Method and 95% Confidence

starch*OLE	N	Mean	Grouping
5 no	2	1.81	A
5 yes	2	1.80	A
0 yes	2	1.77	A
10 no	2	1.76	A
0 no	2	1.76	A
10 yes	2	1.74	A
15 no	2	1.61	B
15 yes	2	1.59	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

OLE	N	Mean	Grouping
no	8	1.74	A
yes	8	1.73	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
5	4	1.81	A
0	4	1.76	A B
10	4	1.75	B
15	4	1.60	C

Means that do not share a letter are significantly different.

Moisture content

Grouping Information Using the Tukey Method and 95% Confidence

starch*OLE	N	Mean	Grouping
0 no	2	18.97	A
0 yes	2	16.70	B
5 no	2	12.81	C
5 yes	2	11.27	C D
10 no	2	10.73	D E
15 no	2	9.33	E F
15 yes	2	8.44	F
10 yes	2	8.43	F

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

OLE	N	Mean	Grouping
no	8	12.96	A
yes	8	11.21	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
0	4	17.83	A
5	4	12.04	B
10	4	9.58	C
15	4	8.89	C

Means that do not share a letter are significantly different.

Solubility

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
0	4	41.53	A
5	4	36.97	B
10	4	35.15	B
15	4	24.18	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

OLE	N	Mean	Grouping
no	8	37.29	A
yes	8	31.62	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch*OLE	N	Mean	Grouping
0 no	2	44.06	A
5 no	2	40.50	A B
0 yes	2	39.01	B C
10 no	2	35.82	B C D
10 yes	2	34.48	C D
5 yes	2	33.45	D E
15 no	2	28.81	E
15 yes	2	19.56	F

Means that do not share a letter are significantly different.

Swelling Degree

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
15	4	149.90	A
10	4	140.42	A B
5	4	133.887	A B
0	4	121.656	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

OLE	N	Mean	Grouping
no	8	143.634	A
yes	8	129.302	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch*OLE	N	Mean	Grouping
15 no	2	161.713	A
10 no	2	148.135	A B
5 no	2	140.093	A B
15 yes	2	138.100	A B
10 yes	2	132.708	A B
5 yes	2	127.681	A B
0 no	2	124.594	B
0 yes	2	118.718	B

Means that do not share a letter are significantly different.

Opacity

Grouping Information Using the Tukey Method and 95% Confidence

starch	N	Mean	Grouping
0	4	8.91800	A
5	4	6.05406	B
10	4	5.86259	B
15	4	4.61917	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

oli	N	Mean	Grouping
yes	8	6.73072	A
no	8	5.99619	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

starch*oli	N	Mean	Grouping			
0 yes	2	9.05685	A			
0 no	2	8.77914	A			
5 yes	2	6.76805		B		
10 yes	2	6.07764		B	C	
10 no	2	5.64754		B	C	
5 no	2	5.34008			C	D
15 yes	2	5.02033			C	D
15 no	2	4.21801				D

Means that do not share a letter are significantly different.