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Development of Oleuropein Incorporated Chitosan Films for Antioxidant Active Food Packaging Applications

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Abstract

Oleuropein is the major phenolic component of olive leaf extract. In the present study, oleuropein was incorporated into chitosan films, and the physical properties and antioxidant activity of the films were determined. The chitosan-to-oleuropein ratio was arranged as 1:1, 1:0.5, 2:1, and 2:0.5. Physical properties including moisture content, density, solubility, water vapor permeability, color, and opacity were measured. The results showed that the addition of oleuropein improved the water vapor barrier and decreased the solubility of chitosan films. The oleuropein-added films had the same density and moisture content as chitosan films. Oleuropein incorporation resulted in higher opacity and b* values, whereas a* and L* values decreased. Chitosan films with oleuropein showed strong antioxidant activity. Films at chitosan/oleuropein ratio as 1:1 could be good candidate for active packaging material due to exhibiting good water vapor barrier, opacity, and antioxidant property.

1. Introduction

Olive leaves are a significant waste from olive oil production, and they can be considered a cheap and rich source of phenolic compounds. In general, olive leaves contain about 30 phenolic compounds, which are phenolic acids (gallic acid, coumaric acid, ferulic acid, syringic acid, caffeic acid, vanillic acid), phenolic alcohols (tyrosol, hydroxytyrosol), flavonoids (luteolin, apigenin, quercetin, cyanidin), secoiridoids (oleuropein, verbascoside, ligroside) and lignans [1]. Oleuropein is a heterosidic ester of β-glucosylatedelenolic acid and hydroxytyrosol. C25H32O13 is the molecular formula of oleuropein, and its molecular weight is 540.51 g mol-1 [2]. Oleuropein is mainly present in olive tree leaves and olives. It is also present in small amounts in olive oil and is responsible for the bitter taste of olives and olive oil [3].. The studies demonstrated significant health benefits, including the prevention of coronary heart disease, neurodegenerative diseases, and cancer [4]. Oleuropein has shown strong antimicrobial properties in several studies. Sudjana et al. stated that oleuropein, as the main component of leaf extract, showed antimicrobial activity against Helicobacter pylori, Campylobacter jejuni, and Staphylococcus aureus [5]. In a similar study, oleuropein was found to have inhibitory effect especially on Gram(+) bacteria [6].

In recent years, due to health and environmental concerns about the use of petrochemical-based plastics, biopolymer-based packages have received great attention for replacing plastic food packaging. Carbohydrate polymers such as starch, pectin, cellulose derivatives, and chitosan could be good alternatives for biopolymer-based packaging materials [7]. Among them, chitosan, a cationic polysaccharide consisting of β -1,4-linked D-glucosamine and N-acetyl-d-glucosamine, has been shown to be a potential packaging material due to being nontoxic, biodegradable, biocompatible and also having antimicrobial properties [8]. Showing

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antimicrobial properties makes chitosan as film matrix for active food packaging design to minimize food deterioration and extend the shelf life of foods. To enhance the antimicrobial activity of chitosan films, extracts of phenolic compounds such as grape seed extract [9], onion skin extracts [10], and rich cactus pear extract [11], have been commonly used. Moreover, essential oils like tea tree oil [12], lemon, thyme, and cinnamon oils [13], and basil oil [13], were used to increase the antimicrobial activity of chitosan films to be used as active packaging materials. On the contrary, the antioxidant activity of chitosan films is almost negligible, so it is needed to fortify the antioxidant activity of chitosan films to minimize oxidation of food during storage. Although synthetic antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ) etc.) show high stability and low cost, due to safety and health concerns, consumers do not prefer packages having synthetic antioxidants [14]. Therefore, natural antioxidants have received great attention as a way to replace synthetic ones. The incorporation of phenolic compounds, in particular, has been found to be effective in providing strong antioxidant activity for chitosan films. In the study of Kadam et al., pine needle extract was incorporated into chitosan films, and a significant increase in the antioxidant activity of chitosan films was observed [15]. In a similar study, blueberry and blackberry pomace extracts were used as active agents, and phenolic compounds incorporated into chitosan films showed strong antioxidant activity [16].

The main aim of this study is to produce oleuropein incorporated chitosan films to be used as biodegradable active packaging materials. The effects of different oleuropein and chitosan concentrations on the physical (density, moisture content, water solubility, water vapor permeability, opacity, and color) and antioxidant properties of chitosan-based films were evaluated.

2. Material and Method

2.1. Materials

Chitosan was bought from Sigma-Aldrich Chemie Gmbh (Darmstadt, Germany), and oleuropein powder was provided by Kale Naturel Ltd. Şti. Glycerol (≥%99), 2,2-Diphenyl-1-picrylhydrazyl, Folin-Ciocalteau reagent, sodium carbonate) were purchased from Sigma-Aldrich Chemie Gmbh (Darmstadt, Germany).

2.1. Methods

2.2.1 Film preparation

To prepare chitosan solutions (1% and 2% (w/v)), 1 g and 2 g of chitosan were dissolved in 100 ml of acetic acid solution (1% v/v). Glycerol was added to the prepared solutions by arranging chitosan: glycerol ratio 2:1 (w/w). The solutions were stirred for 2 hours with a magnetic stirrer. %5 and %2.5 (w/v) oleuropein were dissolved in ethanol/water (80/20) solution and the slurry was centrifuged at 3500 rpm for 10 minutes to remove unsoluble parts. To obtain a chitosan/oleuropein ratio as 1:1, 1:0.5, 2:1, 2:0.5, oleuropein was added to chitosan solutions. 15 ml of solutions were cast over petri plates for 48 h at 25°C. Before physical analyses, the films were stored in a desiccator at 52% and 20°C for conditioning. The oleuropein incorporated chitosan films were named as Ch1Ole1, Ch1Ole0.5, Ch2Ole1, Ch2Ole0.5. 1% and 2% chitosan films were prepared and labelled as Ch1 and Ch2, respectively.

2.2.2 Thickness of films

The film thickness was measured using a digital micrometer (Dial thickness gauge No. 7301, Mitutoyo Co. Ltd., Tokyo, Japan). Six random readings from three films were taken.

2.2.3. Density, moisture content and solubility of films

The initial weight of films being at 2 cm × 2 cm was recorded (W_1). Until the samples reached a constant weight (W_2), the films were dried in an oven at 105 °C. The density was calculated from Eqn. [1];

$$\boldsymbol{Density} = \frac{\boldsymbol{W_2}}{\boldsymbol{Area \times Thickness}} \quad (1)$$

Moisture content (%, wet basis) was found from the Eqn. [2];

$$Moisture\ content\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

The dried films (W_2) were put in 30 ml of distilled water at room temperature and waited for 24 h. Then, the undissolved parts were dried in an oven set at 105°C for 24 h, and the weight of the dried undissolved parts was recorded (W_3). Solubility was calculated by Eqn. [3];

$$Solubility\ (\%) = \frac{W_2 - W_3}{W_2} \times 100 \quad (3)$$

2.2.4. Color of films

The film color was determined using a Chroma Meter CR400 colorimeter (Konica Minolta, Inc., Japan). L*(lightness), a*(redness- greenness), b* (yellowness-blueness) were used to characterize the color of films, and the total color difference ΔE was calculated using the following Eqn. [4];

$$\Delta E = \sqrt{(L_0{}^* - L^*)^2 + (a_0{}^* - a^*)^2 + (b_0{}^* - b^*)^2} \quad (4)$$

where $L_0{}^*$, $a_0{}^*$ and $b_0{}^*$ are the color measurements of $BaSO_4$ plate.

2.2.5. Opacity of films

The film opacity was measured at 600 nm using a UV–visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Opacity values were determined according to the method of Aydogdu et al. with Eqn. [5]; [17];

$$Opacity = \frac{A_{600}}{x} \quad (5)$$

where A_{600} is the absorbance at 600 nm and x is the thickness value of films (mm). Greater opacity values indicate lower transparency of the films. Three pieces were taken from each film for measurement; three replicates were measured for each formulation.

2.2.6. Water vapor permeability of films (WVP)

WVP of films was determined with the modified version of ASTM E-96 described by Aydogdu et al. [17].The custom-built test cups were used in this experiment. To provide the films with 100% RH, 30 mL of distilled water was used to fill the cups. The cups were placed in a desiccator at 40% RH using silica gel. The cups were weighed at 3 h intervals. The water vapor transmission rate (WVTR) was calculated from the slopes of weight loss data vs. time. Then the WVP values were calculated by using Eqn. [6];

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

where x is the thickness of the films (m), S is the saturated water vapor pressure (Pa) at measured temperature, R_1 and R_2 are the relative humidity inside the cups and desiccator, relatively. The measurements were performed in duplicate.

2.2.7. DPPH radical-scavenging activity

The modified method described by Aydogdu et al. [18] was applied to determine the DPPH radical-scavenging activity. Pieces of films (0.1 g) and 10 ml of ethanol/water (80/20) were mixed. 3.9 mL $DPPH^{\cdot}$ radical solution was added to 100 µl sample solutions in the dark at room temperature. After 1 hour, the absorbance of mixtures was measured at 517 nm by a spectrophotometer (UV 1800, Shimadzu, Columbia, USA). The antioxidant activity (%AA) of the films was defined by Eqn. [7] ;

$$\text{DPPH scavenging activity (AA)}(\%) = \frac{A_{control} - A_{film}}{A_{control}} \times 100 \quad (7)$$

where $A_{control}$ and A_{film} are the absorbance values of the DPPH solution without and with the presence of the sample solutions.

2.2.8. Total phenolic content (TPC) of films

The TPC of films was determined by the modified Folin–Ciocalteau method [19]. To extract phenolic compounds from the film, about 0.1-0.2 g of the film was put in 10 ml of ethanol water mixture (4:1 v/v). According to absorbance values, samples were diluted, and 1 ml of sample and 2.5 ml of 0.2 N Folin-Ciocalteau reagent were mixed. Then, the mixture was kept in dark for 5 min. 2 ml of a 75 g/L sodium carbonate solution were added to the mixture. 1 hour in dark at room temperature was waited. The absorbance values of mixtures at 760 nm were measured by a spectrophotometer (UV 1800, Shimadzu, Columbia, USA), and TPC was determined by Eqn. [8] ;

$$\text{TPC}\left(\text{mg}\frac{\text{GAE}}{\text{g}}\text{film}\right) = \frac{C \times V \times D}{W_S} \quad (8)$$

where C is the concentration corresponding to the measured absorbance value from the calibration curve

(mg/L), V is the volume of the solution (L), D is the dilution rate, and Ws is the weight of the film (g).

2.2.9 Statistical analysis

Data were analyzed by MINITAB (version 16, State College, PA, USA). An analysis of variance (ANOVA) was applied, and Tukey's Multiple Comparison Test was used for the identification of the significance of differences among values ($p \leq 0.05$).

3. Results and Discussion

3.1 Moisture Content, Thickness, Density and Solubility

Table 1 represents the thickness, solubility, moisture content, and density of films. The thickness values were within the range of 0.036-0.042 mm, and no significant difference between the films was observed ($p > 0.05$). While the density of oleuropein incorporated chitosan films lay within the range of 1.21-1.34 g/cm3, the density values of chitosan films (1% and 2%) were 0.55 and 0.62 g/cm3. Although there is no significant difference in the density of films with varying amounts of oleuropein, the addition of oleuropein increased the density of chitosan films significantly. The moisture content of both chitosan and oleuropein incorporated chitosan films was in the range of about 16%-19% which is similar to phenolic acid incorporated chitosan films [8],[10]. The chitosan films containing oleuropein showed a considerable reduction ($p \leq 0.05$) in water solubility as compared to the control chitosan films. The interaction between oleuropein and chitosan chains reduced the interaction of water with the chitosan. A similar trend was found by Rambabu et al. [20], which showed the addition of mango leaf extract decreased the solubility of chitosan from 27% to 17% (wb).

3.2 WVP

The WVP value of films is an indication of moisture transfer between food and environment, so it is a critical parameter that affects the shelf lives of foods. WVP values of films are shown in Table 1. The addition of oleuropein resulted in films with a lower WVP, which was desirable to minimize moisture transfer. The reason could be related to filling of the gaps existing in the matrix with the help of oleuropein [20]. This is consistent with the density results, and the incorporation of oleuropein into the chitosan film caused denser films (Table 1). Moreover, oleuropein as a phenolic compound interact with reactive groups of chitosan by hydrogen or covalent bonding and these interactions could decrease the amount of available hydrogen groups to obtain hydrophilic bonding with water [21]. Therefore, the affinity of chitosan films towards the water could be reduced by oleuropein addition. Similar reducing effects of phenolic compounds on WVP values of films were observed in several studies, such as propolis [22], blueberries [16], tea extract [8], etc.

3.3 Color and Opacity

The color parameters (L*, a*, b*, and ΔE*) of films were represented in Table 2. It is obviously seen that oleuropein addition decreased L* values of chitosan films which is an evidence of obtaining darker films. Oleuropein incorporation resulted in lower a* and higher b* values. a* value represents greenness (-) and redness (+) and b value represent yellowness (+) and blueness (-). The color of oleuropein as olive leaf extract is greenish yellow, so chitosan films including oleuropein showed a* values in the range of -3.13 and -4.12. Moreover, as the oleuropein amount increased, the b* values of chitosan films increased significantly ($p \leq 0.05$). Although chitosan films were almost colorless, a significant ΔE* increase was observed by adding oleuropein into chitosan films. ΔE* values of Ch_Ole films were in the range of 23.39- 34.99. If ΔE* value is greater than 5, the color change of films can be detected by observers [23] so the color change of oleuropein incorporated chitosan films was easily visible.

The transparency of films affects customer acceptance, so it is a crucial property of packaging material. Although transparent packaging makes it possible to see the product directly, it is not appropriate for the packaging of photo-sensitive foods. The opacity values of films are shown in Table 2. Chitosan films having 1.15 and 1.52 A mm−1 opacity were almost transparent. When oleuropein added to films, the opacity values reached up to 5.8 A mm−1. The addition of oleuropein resulted in light scattering and yielded in higher opacity. Similar studies demonstrated that phenolic acid addition increased opacity values for green tea extract [21], pomegranate peel extract [24], black soybean seed coat extract [25], apple peel polyphenols [26] etc.

Table 1. Thickness, moisture content, solubility, density and water vapor permeability (WVP) values of chitosan and oleuropein incorporated chitosan films

Films	Thickness(mm)	Moisture Content (%)	Solubility (%)	Density (g/cm^3)	WVP x 10^{10} ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$)
Ch1Ole1	0.042±0.002^a	16.38±0.36^a	27.43±2.33^b	1.34±0.13^a	1.401±0.012^b
Ch1Ole0.5	0.036±0.001^a	17.05±0.06^a	26.64±0.19^b	1.21±0.12^a	1.394±0.013^b
Ch2Ole1	0.038±0.004^a	18.92±0.56^a	29.13±1.74^b	1.22±0.11^a	1.423±0.024^b
Ch2Ole0.5	0.036±0.001^a	18.43±0.74^a	28.33±1.77^b	1.30±0.11^a	1.442±0.036^b
Ch1	0.040±0.003^a	18.32±0.20^a	32.87±3.31^a	0.55±0.02^b	2.729±0.048^a
Ch2	0.040±0.002^a	17.55±0.38^a	32.33±2.41^a	0.62±0.02^b	2.616±0.016^a

Different letter superscripts in the same line indicate a statistically significant difference ($p \leq 0.05$). ± indicates standard error.

Table 2. Color parameters and opacity values of chitosan and oleuropein incorporated chitosan films

Films	L*	a*	b*	ΔE*	Opacity (A mm^{-1})
Ch1Ole1	82.29±1.48^b	-4.12±0.55^b	34.52±1.70^a	34.99±3.41^a	4.36±0.25^a
Ch1Ole0.5	83.24±1.48^b	-3.13±0.22^b	27.17±1.63^b	24.84±2.54^b	5.00±0.69^a
Ch2Ole1	81.94±0.64^b	-3.72±0.16^b	31.05±2.08^a	32.03±2.09^a	5.46±0.55^a
Ch2Ole0.5	84.07±1.38^b	-3.41±0.28^b	23.79±1.20^b	23.39±2.04^b	5.80±0.25^a
Ch1	91.82±0.22^a	-0.65±0.03^a	1.15±0.23^c	6.00±0.57^c	1.15±0.11^b
Ch2	91.89±0.42^a	-0.54±0.10^a	0.56±0.11^c	5.57±0.18^c	1.52±0.12^b

Different letter superscripts in the same line indicate a statistically significant difference ($p \leq 0.05$). ± indicates standard error

3.4 TPC and Antioxidant Activity

Table 3 shows the total phenolic content and antioxidant activity of films. As shown in Table 3, oleuropein incorporation significantly increased the total phenolic content of chitosan films, as expected. Ch1Ole1 films showed the highest total phenolic content (35.10±1.44 mg GAE/g film) with a chitosan to oleuropein ratio of 1:1 w/w. Oleuropein is one of the dominant phenolic acids in olive leaves and fruits [27]. Phenolic compounds are potential antioxidants, so oleuropein added chitosan films showed antioxidant activity between 67.21 %- 98.25. In fact, chitosan films showed meager antioxidant activity with about 17-18%. [20] stated that chitosan polysaccharide chain enhanced antioxidant activity. However, these values are not enough to suggest films as an active packaging material. Oleuropein, as an antioxidant source, enhanced the antioxidant activity of chitosan films. Antioxidant activity of films was directly correlated to their total phenolic contents. Therefore, due to having the highest total phenolic content, Ch1Ole1 films showed the strongest antioxidant activity at nearly 100%. Thus, Ch1Ole1 films could be a good option for the antioxidant food package.

Table 3. TPC and DPPH activity of chitosan and oleuropein incorporated chitosan films

Films	TPC (mg GAE/g film)	DPPH activity (%)
Ch1Ole1	35.10±1.44^a	98.25±1.50^a
Ch1Ole0.5	26.02±1.31^b	84.72±3.46^b
Ch2Ole1	17.84±0.95^c	83.66±1.81^b
Ch2Ole0.5	12.60±1.43^d	67.21±2.74^c
Ch1	1.90±0.07^e	18.45±2.20^d
Ch2	2.45±0.16^e	17.85±0.38^d

Different letter superscripts in the same line indicate a statistically significant difference ($p \leq 0.05$). ± indicates standard error.

4. Conclusion

Oleuropein is one of the major phenolic compounds in olives and olive leaves. With the addition of oleuropein, the thickness and moisture content of films were not affected, but water solubility and water vapor permeability decreased significantly, suggesting that the water barrier property of the film was improved. Oleuropein incorporation reduced the lightness and increased the greenness and blueness of the chitosan films. Besides, more opaque films were obtained by increasing oleuropein content. The total phenolic content of chitosan films increased as the

amount of oleuropein increased, representing significant antioxidant activity up to nearly 100% when combined with the chitosan films. Conclusively, enhanced water vapor permeability, water solubility, opacity, and strong antioxidant activity indicate that chitosan films containing oleuropein can be a promising alternative to biodegradable active packaging materials for shelf-life extension of foods.

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Contributions of the authors

Ayca Aydogdu Emir: conceptualization, supervision, methodology, investigation, writing—original draft; Fatmagül Kaya: methodology, investigation, writing—original draft, visualization.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

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