Turkish Journal of Science & Technology
18(1), 87-95, 2023

Research Paper
https://doi.org/10.55525/tjst.1187542

Assessment of Elenolic Acid Incorporation on Physical Properties of Chitosan Films to be Used as Active Packaging Material

Ayça AYDOĞDU EMİR [1*], Osman UÇKUN [2]

[1] Department of Food Technology, Faculty of Canakkale Applied Sciences, Canakkale 18 Mart University, Canakkale, Turkey
[2] Department of Food Technology, Faculty of Canakkale Applied Sciences, Canakkale 18 Mart University, Canakkale, Turkey
[*1] ayca.aydogdu@comu.edu.tr, [2] uckunosman95@gmail.com

(Geliş/Received: 11/10/2022; Kabul/Accepted: 08/02/2023)

Abstract: There is growing interest in biodegradable active packaging materials to extend shelf lives of food by retarding deteriorative reactions. The objective of this study was to fabricate active packaging films made from elenolic acid and chitosan. Elenolic acid is one of the phenolic compounds of olive leaves. Different amount of elenolic acid (%2.5 and %5 w/v) was incorporated into chitosan films (%1 and 2% w/v). The physical properties (density, moisture content, solubility, water vapor permeability, opacity, and color), total phenolic content and antioxidant activity were investigated. While elenolic acid addition did not affect the moisture content of chitosan films and the density, opacity, a* and b* values increased significantly ($p \leq 0.05$). Elenolic acid incorporation reduced the water vapor permeability of chitosan films by 25%. Correlated to total phenolic content of the films, antioxidant activity of films reached up to % 85. Elenolic acid added chitosan films exhibited good water vapor barrier properties, opacity and antioxidant activity indicating that they could be developed as biodegradable active food packaging material for the food industry.

Key words: Elenolic acid, chitosan, active package, antioxidant activity, water vapor permeability.

Elenolik Asit Katılmasının Aktif Ambalaj Malzemesi Olarak Kullanılacak Kitosan Filmlerinin Fiziksel Özellikleri Üzerine Etkisinin Değerlendirilmesi

Öz: Bozunma reaksiyonlarını geciktirerek gıdaların raf ömrünü uzatmak için biyolojik olarak parçalanabilen aktif ambalaj malzemelerine artan bir ilgi vardır. Bu çalışmanın amacı, elenolik asit ve kitosandan yapılan aktif ambalaj filmleri üretmektir. Elenolik asit, zeytin yapraklarının fenolik bileşiklerinden biridir. Kitosan filmlere (%1 ve %2 w/v) farklı miktarlarda elenolik asit (%2.5 ve %5 w/v) eklenmiştir. Filmlerin fiziksel özellikleri (yoğunluk, nem içeriği, çözünürlük, su buharı geçirgenliği, opaklık ve renk), toplam fenolik içerik ve antioksidan aktivite araştırılmıştır. Elenolik asit ilavesi kitosan filmlerin nem içeriğini etkilemezken yoğunluk, opaklık, a* ve b* değerleri önemli ölçüde artmıştır ($p \leq 0.05$).. Elenolik asit eklenmesi, kitosan filmlerinin su buharı geçirgenliğini %25 oranında azaltmıştır. Toplam fenolik içeriği ile ilişkili olarak, filmlerin antioksidan aktivitesi %85'e kadar çıkmıştır. Elenolik asit katkılı kitosan filmler, iyi su buharı bariyer özellikleri, opaklık ve antioksidan aktivite sergileyerek, gıda endüstrisi için biyolojik olarak parçalanabilen aktif gıda ambalaj malzemesi olarak geliştirilebileceğini göstermektedir.

Anahtar kelimeler: Elenolik asit, kitosan, aktif paket, antioksan aktivite,su buharı geçirgenliği.

1. Giriş

Due to rising consumer awareness of sustainability and health concern for using plastic food packaging, the growing interest in the development of biopolymer-based packages has increased. In general, to produce edible and biodegradable films, biopolymers include polysaccharides, protein and lipids [1]. Moreover, to extend the shelf life of foods and maintain food quality, active packaging systems are developed. One of the main reasons of food deterioration is oxidation and to improve the quality of oxidation sensitive foods, antioxidant incorporated food packaging technology is an effective alternative [2]. The common synthetic antioxidants thar are commonly used in food industry are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). However, due to growing health concern, there is great demand to replace synthetic antioxidants with natural ones. In general, tocopherol, plant extracts, and essential oils from herbs and spices are widely used natural antioxidants [3]. Olive leaves are significant waste in olive oil production. Olive leaves are rich source of phenolic compounds so olive leaf extracts are good candidates for active packaging

[*] Corresponding author: ayca.aydogdu@comu.edu.tr. [1] 0000-0003-3877-9200 , [2] 0000-0001-9454-3695

designs [4]. Oleuropein is the main component of olive leaf extract, and it is a complex phenolic compound olive tree leaves [5]. Elenolic acid is the form of chemical hydrolysis of oleoside methylester, oleuropein and ligstroside and it is naturally found in olive oil linked to tyrosol and hydroxytyrosol [6]. The studies of Briante et al. (2002) [7] and Thiealmann et al. [8] showed the antioxidant and antimicrobial activities of elenolic acid derivatives. In this study, elenolic acid was used as active compound to produce biodegradable active packaging material. Chitosan is one of the most commonly used biopolymer due to its non-toxicity, biodegradability, biocompatibility. Chitosan is a cationic polysaccharide obtained by deacetylation of chitin and consists of β-(1-4)-2- acetamido-α-glucose and β -(1-4)-2-amino- α -glucose units [9]. Compared to other biopolymers used for edible films, chitosan shows excellent film forming characteristics such as mechanical performance and good barrier properties [10]. Moreover, owing to its antimicrobial activity, chitosan is great option to produce antimicrobial packaging [9] [11]. To improve antimicrobial activity of chitosan films, several phenolic compounds [12], [13] and essential oils [14][15] were incorporated. In contrast to antimicrobial activity, chitosan does not show significant antioxidant activity. Therefore, it is important to enhance of antioxidant activity of chitosan films to be used for biopolymer-based antioxidant packages. Thus, in several studies, extracted phenolic compounds such as tea polyphenols [9], onion skin [16], apple peel [12], black soybean seed coat [17] were incorporated into chitosan films and significant increase in antioxidant activity was observed. To the best of our knowledge, no study in the literature examines the use of elenolic acid for active packaging materials. The objective of this study is to develop environmentally friendly, biodegradable films from chitosan incorporated elenolic acid to be used as active packaging material. Within this scope, total phenolic content, antioxidant activity and physical properties, including density, moisture content, water solubility, water vapor permeability, opacity and color of the films were investigated.

2. Materials and Methods

2.1 Materials

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

2.2 Methods

2.2.1 Film preparation

1% and 2% (w/v) chitosan solutions were prepared by dissolving 1 g and 2 g of chitosan in 100 ml of acetic acid solution (1% v/v). 0.5% (w/v) glycerol was added to the prepared solution as a plasticizer and the mixture was stirred for 2 hours with a magnetic stirrer. %5 and %2.5 (w/v) elenolic acid was dissolved by ethanol/water (80/20) solution. Then, to remove insoluble particles, the slurry was centrifuged (Nüve, NF 800, Turkey) at 3500 rpm for 10 minutes. Chitosan and elenolic acid solutions were mixed to get chitosan/elenolic acid ratio as 1:1, 1:0.5, 2:1, 2:0.5. Solutions of 15 ml were poured into LDPE petri plates and dried at room temperature (25°C, 50%RH) for 24 h. For conditioning, the films were stored in a desiccator (52%, 20°C) before analysis. The films were labelled as Ch1Ele1, Ch1Ele0.5, Ch2Ele1, Ch2Ele0.5. Control films were 1% and 2% chitosan films named as Ch1 and Ch2, respectively.

2.2.2 Thickness of films

To measure thickness values of films, a hand-held micrometer (Dial thickness gauge No. 7301, Mitutoyo Co. Ltd., Tokyo, Japan) was used. Six measurements from three films were done.

2.2.3. Density, moisture content and solubility of films

The films were cut into pieces (2 cm × 2 cm), and their initial weight was recorded (W_1). Films were dried until the samples reached a constant weight (W_2) by placing them inside an oven set to 105 °C. Density was calculated from the Eq. 1;

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

Moisture content (%, wet basis) was found from the Eq. 2;

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

The dried films (W_2) were immersed in 30 ml of distilled water at room temperature and and kept there for 24 h. Then, the undissolved remnants were dried in a 105°C oven for 24 h, and the final weight was recorded (W_3). Solubility was calculated by Eq. 3;

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

2.2.4. Opacity of films

Light transmittance of the films was measured with a UV–visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Opacity values were calculated from the method described by (Aydogdu et al., 2018) [1] with Eq. 4 ;

$$Opacity = \frac{A_{600}}{x} \quad (4)$$

where A_{600} is the absorbance at 600 nm and x is the film thickness (mm). Three pieces were taken from each film for measurement; three replicates were measured for each formulation.

2.2.5. Color of films

To investigate color of the films, a color reader Chroma Meter CR400 (Konica Minolta, Inc., Japan) was used. The values were expressed in the CIE L*(lightness), a*(redness- greenness), b* (yellowness-blueness) color space by the help of Eq. 5;

$$\Delta E = \sqrt{(L_O{}^* - L^*)^2 + (a_O{}^* - a^*)^2 + (b_O{}^* - b^*)^2} \quad (5)$$

where L_0^*, a_0^* and b_0^* are the color measurements of control films.

2.2.6. Water vapor permeability of films (WVP)

WVP of chitosan/elenolic acid films was measured with the modified version of ASTM E-96 described by (Aydogdu et al., 2018) [1].The custom-built test cups are cylindrical polyacetal (Delrin) with a 40 mm internal diameter. To provide the films to 100% RH, 35 mL of distilled water were filled into cups. The cups were placed in a desiccator at 25-30 % RH by using silica gel. The cups were weighed at 2 h intervals. The Water vapor transmission rate (WVTR) was calculated from the regression analysis of weight loss data vs. time. Then the WVP values were calculated by using Eq. 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. Total phenolic content of films

Total phenolic contents of films were determined by the modified Folin–Ciocalteau method [18]. Briefly, about 0.1 g sample was dissolved in 10 ml ethanol/water (80/20) to extract phenolic compounds of films. Diluted 1 ml of sample was mixed with 0.2 N Folin-Ciocalteau reagent and kept in dark for 5 min and then, 2 ml of 75 g/L sodium carbonate solution was added. After standing for 1 hour in dark at room temperature, the absorbance at 760 nm was measured by a spectrophotometer (UV 1800, Shimadzu, Columbia, USA) and total phenolic content (TPC) was calculated as follows;

$$\text{TPC (mg GAE/g film)} = \frac{C \times V \times D}{W_S} \quad (7)$$

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 W_s is the weight of the film (g).

2.2.8. DPPH radical-scavenging activity

The modified method described by Aydogdu et al. 2019 [19] was applied to determine the DPPH radical-scavenging activity. Firstly, about 0.1 g film was dissolved in 10 ml ethanol/water (80/20). After the removal of undissolved parts, solutions at 100 µl were mixed with 3.9 mL DPPH˙ radical solution and kept in dark place for 1 h to complete reaction between DPPH solution and phenolic compounds. Absorbance value of samples was measured at 517 nm by a spectrophotometer (UV 1800, Shimadzu, Columbia, USA). The antioxidant activity (%AA) of the films were defined by Eq. 8 ;

$$\text{DPPH scavenging activity (\%)}=\frac{A_{control}-A_{film}}{A_{control}}\times 100 \quad (8)$$

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

2.2.9 Statistical analysis

All experiments were performed in duplicate. Analysis of variance (ANOVA) was applied by MINITAB (version 16, State College, PA, USA). If significant differences were found, Tukey's Multiple Comparison Test was used for comparisons (p≤0.05).

3. Results and Discussion

3.1 Moisture content, density and solubility

Thickness, moisture content, water solubility and density of the films are shown in Table 1. Thickness values were within the range 0.072–0.084 mm. The fact that there was no significant difference between the samples (p>0.05) represented that elenolic acid addition did not affect the average thickness. The density values (Table 1) lay within the range 1.28-1.48 g/cm^3 with no statistically significant difference between the formulations (P>0.05). However, it is obviously seen that elenolic acid addition increase the density of chitosan films, significantly. Similar density range (1.132-1.231) were observed with the addition of tea polyphenols into chitosan films [9]. Moisture content of films were in the range of 14.75-18.65% and no significant difference was observed by the addition of elenolic acid. Similarly, Kocakulak et al. 2019 [20] found no difference in moisture content of chickpea flour films with gallic acid addition. Although elenolic acid incorporation did not affect solubility of Ch1 film, Ch2's solubility decreased significantly. Interaction between elenolic acid and the polysaccharide chain of chitosan decreased the available amino and hydroxyl groups of chitosan to interact with water. This resulted in significant decrease in the solubility of chitosan films [21]. Similar decrease in solubility of chitosan films after apple peel polyphenol incorporation observed in the study of Riaz et al.2018 [13] and the reason was stated that phenolic compounds might interact with chitosan molecules with hydrogen bonding and this might decrease the interactions between water molecules and chitosan.

Table 1. Physical properties of films

Film	Moisture Content (%)	Thickness (mm)	Solubility (%)	Density (g/cm^3)
Ch1Ele1	15.94±0.25^a	0.081±0.004^a	33.79±1.01^a	1.33±0.31^a
Ch1Ele0.5	18.65±1.66^a	0.072±0.003^a	32.13±0.31^a	1.48±0.29^a
Ch2Ele1	15.73±1.08^a	0.084±0.005^a	22.62±1.38^b	1.38±0.12^a
Ch2Ele0.5	14.75±0.14^a	0.083±0.003^a	20.59±1.69^b	1.28±0.47^a
Ch1	18.45±0.28^a	0.072±0.006^a	33.46±3.36^a	0.70±0.09^b
Ch2	17.85±0.39^a	0.078±0.003^a	33.35±2.32^a	0.56±0.03^b

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

3.2 Color and opacity

Color properties are directly related to film appearance (Fig. 1). The values of L*, a*, b*, and ΔE* were shown in Table 2. As can be seen, elenolic acid addition resulted in darker films and decreased L* values of chitosan films. Moreover, a* and b* values of chitosan films increased significantly with addition of elenolic acid. This is indication of tendency towards redness and yellowness, respectively. The nature color of elenolic acid powder is brownish so elenolic acid incorporated films had lower L* and higher a* and b* values. There are several studies representing phenolic acid addition resulted in films having high a* and b* values [22], [10]. When elenolic acid amount in films increased, significant increase in the total color difference (ΔE*) was observed that is indication of more colored films. In general, the overall color change of films could be distinguished by the naked eye (ΔE > 5) [23]. Thus, the color change of chitosan/elenolic acid films was obviously visible (Figure 1).

Packages are the only way of presenting foods to consumers; the opacity of films is crucial parameter for consumer acceptance. On the other hand, higher opacity value might be desired to pack foods that could easily oxidized due to light catalyzation. Opaque packaging materials could provide against to light [24]. Opacity values of films were shown in Table 2. While opacity of chitosan films was about 1.3-1.9 A mm^{-1}, the opacity values of elenolic acid incorporated films ranged between 5.49±1.02 and 6.45±0.98 A mm^{-1}. Opacity value of commercial polyethylene packaging films were found as 4.26 A mm^{-1} [25] so it can be stated that chitosan/elenolic acid films can be used as packaging material to provide protection against to light. Incorporation of elenolic acid could cause light scattering and reflection so opacity values increased [26]. This resulted in significant protection to packed foods from light that accelerates nutrient losses, oxidation and discoloration [13]. Similar increase in opacity values was observed in studies about phenolic acid incorporated films; extracts from grape skins [27], red cabbage [28], green tea extract [29] etc.

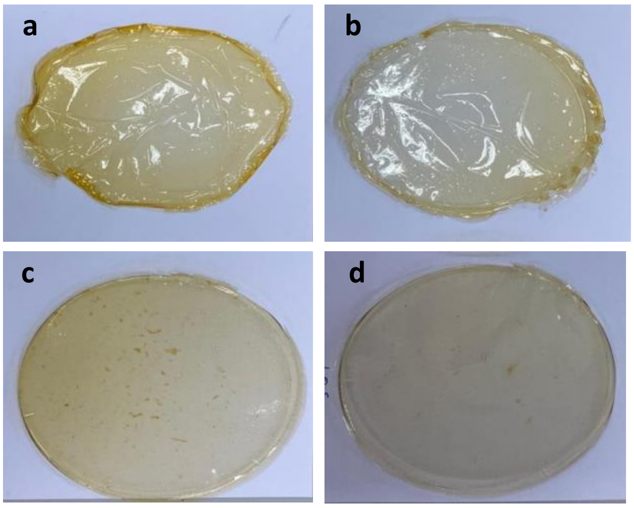

Figure 1. Images of films; a:Ch1Ele1, b: Ch1Ele0.5, c: Ch2Ele1, d: Ch2Ele0.5.

Table 2. Opacity and color parameters of films

Film	Opacity (A mm^{-1})	L*	a*	b*	ΔE*
Ch1Ele1	5.49±1.02^{a}	85.81±1.58^{b}	2.79±0.05^{a}	23.92±1.06^{a}	23.66±1.21^{a}
Ch1Ele0.5	6.45±0.98^{a}	87.35±1.01^{b}	2.41±0.29^{a}	14.45±1.24^{b}	14.16±0.75^{b}
Ch2Ele1	6.41±0.69^{a}	85.01±0.85^{b}	2.57±0.09^{a}	23.14±0.86^{a}	22.72±1.73^{a}
Ch2Ele0.5	5.94±0.63^{a}	86.57±0.41^{b}	2.38±0.11^{a}	16.44±1.21^{b}	15.89±0.99^{b}
Ch1	1.98±0.24^{b}	91.82±0.69^{a}	0.48±0.08^{b}	1.15±0.03^{c}	-
Ch2	1.34±0.12^{b}	91.89±0.22^{a}	0.54±0,11^{b}	1.58±0.12^{c}	-

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

3.3 Total phenolic content and antioxidant activity

The main phenolic compounds of olive leaves and fruits are oleuropein, hydroxytyrosol, tyrosol, coumaric acid, ferulic acid, luteolin, rutin etc.[8]. Oleuropein could be hydrolyzed to elenolic acid, hydroxytyrosol, and oleuropein aglycone [5]. In this study, elenolic acid was added into chitosan films as phenolic compounds. The total phenolic content and antioxidant activity values of chitosan and elenolic acid/chitosan films were presented in Table 3. As can be seen from Table 2, Ch2Ele1 films had the highest total phenolic content with 9.32±0.35 mg GAE/g film followed by Ch1Ele1 films having 7.65±0.09 mg GAE/g film. Films having higher amounts of elenolic acid showed higher total phenolic content. It is known that phenolic compounds are the main source of antioxidant activity. Ch1Ele1, Ch1Ele0.5 and Ch2Ele1 showed the highest antioxidant activity (about %85). As stated before, chitosan shows antioxidant activity and also proved in this study, but pure chitosan films could not affective as active packaging material. However, incorporating elenolic acid, chitosan films could be good candidates for active packaging materials owing to having high antioxidant activity.

3.4 Water vapor permeability (WVP)

Control of moisture transfer between the environment and food which affects the shelf lives of food is the one of the main functions of packaging due to role of water content on deteriorative reactions [30].The WVP values of chitosan and elenolic acid/chitosan films were shown in Table 3. It was observed that regardless of the elenolic acid content, the incorporation of elenolic acid resulted in significant decrease in WVP values ($p \leq 0.05$). While WVP of chitosan films ranged 3.66-3.82x10^{-10} g m^{-1} s^{-1} Pa^{-1}, the WVPs of Ch_Ele films lied within the narrow range of 2.92-2.82x10^{-10}. It is obvious that elenolic acid addition enhanced the water vapor barrier property of chitosan films. One of the reason behind the decrease in WVP of films might the interactions between chitosan and elenolic acid which could decrease the available hydrophilic groups in chitosan and reduce the interactions with water [9]. The other possible reason could be related to higher density of elenolic acid/chitosan films than control chitosan films (Table 1). Elenolic acid incorporation resulted in a dense network so due to the lower space in chitosan matrix, diffusion rate of water through the chitosan films decreased [9]. Thus, WVP of chitosan films enhanced by the addition of elenolic acid. Similar trend was observed phenolic compounds incorporated chitosan films. In study of Kadam et al. 2021 [31] pine needles and in the study of Wang et al. 2022 [16] onion skin phenolic compounds integrated to chitosan films, significant decrease in WVP was observed.

Table 3. Total phenolic content, antioxidant activity and water vapor permeability of the films

Film	TPC (mg GAE/g film)	DPPH activity (% inhibition)	WVP x 10^{10} (g m^{-1} s^{-1} Pa^{-1})
Ch1Ele1	7.65±0.09^{b}	84.90±1.83^{a}	2.86±0.06^{b}
Ch1Ele0.5	4.59±0.30^{d}	83.11±2.71^{a}	2.91±0.05^{b}
Ch2Ele1	9.32±0.35^{a}	85.26±1.15^{a}	2.92±0.06^{b}
Ch2Ele0.5	5.34±0.67^{c}	65.95±2.99^{b}	2.82±0.05^{b}
Ch1	1.22±0.07^{f}	26.71±1.18^{c}	3.82±0.06^{a}
Ch2	1.84±0.08^{f}	25.92±1.09^{c}	3.66±0.02^{a}

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

4. Conclusion

In this study, elenolic acid, component of olive leaf, was used as an active compound to enhance the functional characteristics of chitosan films as an alternative to synthetic antioxidants. Although incorporation of elenolic acid did not affect the density and moisture content of the chitosan films, %2 chitosan films containing different amount of elenolic acid showed lower solubility values which is a desirable property to pack foods having high moisture content. With the addition of elenolic acid, opacity of chitosan films was significantly increased so they can be used to pack light sensitive foods. Due to the nature color of elenolic acid powder, as the elenolic acid amount into films increased, tendency towards redness and yellowness observed. Independent of the elenolic concentrations, elenolic acid addition decreased WVP of chitosan films that is evidence of enhanced water barrier property of the films. Thus, during the shelf life, controlling moisture transfer could be possible. Incorporation of elenolic acid increased total phenolic content and antioxidant activity (almost %85) of chitosan films significantly. Results suggested that elenolic acid added chitosan films could be used as promising alternative to synthetic packaging materials and could also have ability to extend shelf lives of foods. As a future study, the real package system can be design by using elenolic acid incorporated chitosan films and shelf-life extension can be investigated.

Acknowledgment

The research was supported by the Research Fund of the Çanakkale Onsekiz Mart University with Project Number FBA-2021-3573. Ayca Aydogdu Emir: conceptualization, supervision, methodology, investigation, writing—original draft; Osman Uçkun: methodology, investigation, writing—original draft, visualization.

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