Food Packaging and Shelf Life 20 (2019) 100316

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Development of flexible antimicrobial zein coatings with essential oils for the inhibition of critical pathogens on the surface of whole fruits: Test of coatings on inoculated melons

Derya Boyacı[a], Gianmarco Iorio[b], Gozde Seval Sozbilen[a], Derya Alkan[a,1], Silvia Trabattoni[c], Flavia Pucillo[b], Stefano Farris[b,*], Ahmet Yemenicioğlu[a,*]

[a] Department of Food Engineering, Faculty of Engineering, Izmir Institute of Technology, 35430, Gülbahçe Köyü, Urla, Izmir, Turkey
[b] DeFENS, Department of Food, Environmental and Nutritional Sciences, Packaging Division – University of Milan, Via Celoria 2, 20133, Milan, Italy
[c] Department of Materials Science, University of Milano - Bicocca, via R. Cozzi 55, I-20125, Milan, Italy

ARTICLE INFO

Keywords:
Antimicrobial packaging
Edible coating
Edible film
Essential oil
Melon
Zein

ABSTRACT

This study aimed to develop essential oil (EO)-containing antimicrobial coatings for the inhibition of pathogenic bacteria contamination on fruit peels. Incorporation of eugenol (EUG), carvacrol (CAR), and thymol (THY) into films at ≥ 1% (w/w) eliminated the typical brittleness of zein films. However, EUG outperformed CAR and THY in terms of mechanical properties. Films with ≥ 2% (w/w) CAR and THY and ≥ 3% EUG showed clear zones against *L. innocua* and *E. coli* in agar medium at 37 °C. All EO-containing films also inhibited *L. innocua* and *E. coli* inoculated at their surfaces by minimum 3.9 and 2.7 decimal (D) within 1 day at 10 °C. Moreover, 2% EUG-containing zein coatings caused 2–3 decimal reduction in *L. innocua* and *E. coli* counts of inoculated melon surfaces at 10 °C. Unlike the bare zein coatings, flexible EUG-containing films on melons did not show cracking or detachment. Zein films loaded with EUG showed a highly hydrophilic surface. The best oxygen barrier performance was observed for the EUG-richest formulation (i.e., EUG at 3%), and this was attributed to a homogenizing effect of the EO that eventually led to a denser and hole-free network. This work suggested that flexible coatings of zein containing EOs could inhibit pathogens embedded in the rough peel surface of melons.

1. Introduction

Due to increased microbial outbreaks originating from whole fruit and minimally processed fruit products, extensive studies have been conducted to improve pre- and post-harvest hygienic procedures and to develop more effective decontamination methods (And & Yousef, 2001; Izumi, 1999; Ma, Zhang, Critzer, Davidson, & Zhong, 2016; Sapers & Sites, 2003; Siro, Devlieghere, Jacxsens, Uyttendaele, & Debevere, 2006; Ukuku, Bari, Kawamoto, & Isshiki, 2005; Ukuku, Geveke, Chau, Bigley, & Niemira, 2017). It is a well-known scientific truth that crops that grow on the ground are particularly risky, as they are in direct contact with sources of potential pathogenic microbial contaminants (irrigation water, manure or fertilizer, animals, etc.) (Chen, Jin, Gurtler, Geveke, & Fan, 2012; Ma et al., 2016; Sapers & Sites, 2003). An outbreak of listeriosis in the United States of America linked to cantaloupe melons in the year 2011 caused a worldwide alert against fruit origin L. *monocytogenes* infections after it caused 33 deaths, 1 miscarriage and 143 hospitalizations (Centers for Disease Control & Prevention, 2012). Thus, susceptible individuals such as pregnant women, elderly people, and immunosuppressed subjects (Álvarez-Ordóñez, Leong, Hickey, Beaufort, & Jordan, 2015; Vázquez-Boland et al., 2001) have particular concerns regarding deadly L. *monocytogenes* from fruits that grow on the ground. The stem scar and rough peels of melons provide a unique protective environment not only for L. *monocytogenes,* but also for *Escherichia coli* O157:H7 and *Salmonella spp*. (Sapers & Sites, 2003). Processes such as cutting and slicing could easily contaminate the inner edible parts of melons and watermelons (Ma et al., 2016). The contaminated fruits also present a risk of cross-contamination when they are stored in cellars, kitchens, or domestic refrigerators without effective washing or isolation from other food.

Antimicrobial coating is a promising active packaging technology that could be applied to increase the safety of these whole fruits by using natural antimicrobials (Appendini & Hotchkiss, 2002; Gennadios, Hanna,

* Corresponding authors.
E-mail addresses: stefano.farris@unimi.it (S. Farris), ahmetyemenicioglu@iyte.edu.tr (A. Yemenicioğlu).
[1] Current address: Muğla Sıtkı Koçman University, Fethiye Faculty of Health Sciences, Department of Nutrition and Dietetics, Karaçulha, 48300 Fethiye, Muğla, Turkey.

https://doi.org/10.1016/j.fpsl.2019.100316
Received 6 October 2018; Received in revised form 21 January 2019; Accepted 19 March 2019
Available online 27 March 2019
2214-2894/

& Kurth, 1997; Han, 2013; Ouattara, Simard, Piette, Bégin, & Holley, 2000; Quintavalla & Vicini, 2002). Edible materials from cellulose, casein, zein, soy protein, and chitosan could be employed for coating whole fresh fruits and vegetables to suppress their respiration rates, since these materials could form odorless, tasteless, and transparent coatings with desired gas barrier/permeation properties (Park, 1999). Zein is a hydrophobic corn protein fraction obtained as the major co-product of rapidly growing oil and bioethanol industries. This biopolymer is attracting a particular industrial interest as a packaging and coating material (Manley & Evans, 1943; Shukla & Cheryan, 2001; Wang et al., 2007; Selling, Woods, Sessa, & Biswas, 2008) due to its outstanding film-forming ability and solubility in organic solvents (e.g., ethanol), which enables the deposition of the film-forming zein solution simply by dipping, spraying, or brushing. Thus, the desired gas permeability characteristics of zein coatings without antimicrobial agents have been exploited to suppress respiration rates of different fruits, such as mangoes (Gol & Rao, 2014; Hoa, Ducamp, Lebrun, & Baldwin, 2002), apples (Bai, Alleyne, Hagenmaier, Mattheis, & Baldwin, 2003), pear (Scramin et al., 2011), tomato (Park, Chinnan, & Shewfelt, 1994; Zapata et al., 2008), and dates (Mehyar, El Assi, Alsmairat, & Holley, 2014). Zein coatings have also been applied on sugar beet and broccoli seeds to delay their sprouting and germination (Assis & Leoni, 2009). Although zein has excellent film-forming properties, high brittleness and restricted flexibility limit its widespread application as a universal coating material. This problem is more pronounced at low moisture conditions with large-sized and rough-surfaced fruits and vegetables. Many studies have been conducted to plasticize zein films with organic acids, sugars, alcohols, and fatty acids (Lai & Padua, 1997; Lawton, 2004; Kim, Sessa, & Lawton, 2004; Ghanbarzadeh et al., 2006; Sessa, Mohamed, & Byars, 2008; Woods, Selling, & Cooke, 2009). However, it was reported that the most effective plasticizers for zein are phenolic compounds (e.g. pure phenolic compounds, phenolic plant extracts, and essential oils) (Arcan & Yemenicioğlu, 2011; Alkan & Yemenicioğlu, 2016).

The antimicrobial zein films developed have been tested mainly on dairy, fish, meat, and poultry products to inhibit pathogenic bacteria (Janes, Kooshesh, & Johnson, 2002; Lin, Wang, & Weng, 2011; Lungu & Johnson, 2005; Marcos, Aymerich, Monfort, & Garriga, 2007; Ünalan, Korel, & Yemenicioğlu, 2011; Ünalan, Arcan, Korel, & Yemenicioğlu, 2013). However, studies targeting the development of antimicrobial zein coatings against risky pathogens in whole fresh fruit that grows on the ground (such as melons and watermelons) are scarce. Chen et al. (2012) showed the antimicrobial potential of chitosan coatings with natural compounds, such as allyl isothiocyanate and nisin on cantaloupe melons, against a cocktail of different *Salmonella* strains. Ma, Zhang, Critzer, Davidson, & Zhong (2016) also successfully tested antimicrobial activity of chitosan coatings containing a mixture of natural and chemical agents (lauric arginate, cinnamon oil, and ethylenediaminetetra acetic acid) on cantaloupe melons against *L. monocytogenes, Escherichia coli* O157:H7, and *Salmonella enterica.*

In the current study, zein films have been developed using different essential oils, namely carvacrol (CAR), thymol (THY), and eugenol (EUG). The goal of this work was to utilize both the antimicrobial activity of EOs and their excellent plasticizing capacity, as recently discovered by Alkan & Yemenicioğlu (2016). The EO-containing antimicrobial flexible zein coatings would be a solution not only to pathogenic bacterial contamination, but also address the cracking and peeling problems associated with the use of bare (i.e., unloaded) zein coatings in dry conditions. The flexible films were first tested in laboratory media for their antimicrobial effects against *L. innocua* and non-pathogenic *E. coli.* The best-performing films were then characterized for their antimicrobial performance on cold-stored (10°C) inoculated melons and for specific physical properties, including surface morphology, wettability, and oxygen permeability. This article is important because it discloses a non-toxic and environmentally friendly alternative to most common chemical disinfection methods applied to melons in order to counteract recent deadly outbreaks.

2. Materials and methods

2.1. Materials

Zein was obtained from Sigma-Aldrich (St. Louis, MO, USA). Eugenol and carvacrol were obtained from Sigma-Aldrich (CHEMIE GmbH, Germany) while thymol was obtained from SAFC (Germany). All other chemicals were reagent grade. The *Listeria innocua* NRRL-B 33,314 (ATCC 1915) and *Escherichia coli* RSHM 4024 (ATCC 25,922) were from the culture collection of the microbiology laboratory of the Department of Food Engineering at Izmir Institute of Technology. The melon cultivar Crenshaw (Cultivar #1) and cultivar Santa Claus (Cultivar #2) were obtained from Tesco supermarket in İzmir, Turkey.

2.2. Methods

2.2.1. Film-making

The zein film was produced as described in Padgett, Han, and Dawson, (1998). Initially, zein (1.4 g) was dissolved in 8.2 mL of ethanol (96%) by mixing for 25 min at 200 rpm. The beaker was covered with Parafilm® M (Bemis NA, Neenah, WI) to avoid evaporation of ethanol. Then, glycerol (0.4 mL) as plasticizer was added dropwise to the zein solution and mixed for 5 min. The film solution was then heated on a hotplate under continuous stirring until boiling. At this point, mixing was stopped and the film solution was cooled to room temperature. Different essential oils (EUG, CAR and THY) were added to the zein-glycerol solution at different concentrations: 0.5%, 1%, 2%, 3%, 4% or 5% (w/w). The mixtures were then homogenized (Heidolph®, Silent Crusher M, Germany) at 10,000 rpm for 4 min, and 6 g of the homogenate was cast onto glass templates (W × L × H: 8.5 × 8.5 × 0.4 cm^3). The films were finally dried at 25 °C for 24 h using a standard incubator.

2.2.2. Antimicrobial film activity on inoculated agar surface

The antimicrobial activity of EUG, CAR, and THY-containing films was tested in aseptic conditions by the agar diffusion method using *Listeria innocua* and *Escherichia coli* as test microorganisms. Fifteen discs (1.3 cm in diameter) were obtained from the films using a cork-borer under aseptic conditions. During the tests, the discs were placed carefully onto Nutrient Agar in Petri dishes, previously inoculated by spreading the inoculum (0.1 mL). The inoculums were prepared in Nutrient Broth using an overnight culture of bacteria in aerobic conditions at 37 °C. The bacterial counts of inoculums for *L. innocua* and *E. coli* used in tests were 7×10^8 CFU/mL and 2.5×10^7 CFU/mL, respectively. The inoculated Petri dishes containing the film discs were incubated for 24 h and 48 h at 37 °C. The diameter of the clear zones formed around the discs was measured by a micrometer (Chronos®, UK) and the corresponding areas were expressed in mm^2.

2.2.3. Antimicrobial activity of inoculated films

EUG, CAR, and THY-containing films were exposed to UV light under a laminar flow hood for 15 min. The films were then cut into 3 cm × 6 cm pieces using a template and a sterile lancet at aseptic conditions and placed into sterile Petri dishes. Two pieces of films were used for each film type. One side of each film surface was inoculated with 225 µL of *L. innocua* (1×10^7 CFU/mL) or *E. coli* cultures (1×10^7 CFU/mL), which were spread using a sterile glass Drigalski spatula. The inoculums were prepared from overnight cultures of *L. innocua* and *E. coli* in nutrient broth that was incubated at aerobic conditions at 37 °C. An aliquot (1 mL) from each active culture was then transferred to 9 mL of nutrient broth in a tube and incubated at 10 °C for 24 h to ensure proliferation at 10 °C. The cultures were then diluted with 0.1% pepton water to obtain their inoculums with 1×10^7 CFU/mL. The films were kept under a laminar flow hood for 20 min for inoculum absorption from the film surface. The Petri dishes containing the inoculated film were then sealed with Parafilm® M and cold-stored at 10 °C. The microbial load of the films was determined on the freshly-prepared samples (time 0) and after 1 and

7 days. For this purpose, inoculated films (0.4 g) were put into a sterile Erlenmeyer flask using sterile tweezers and diluted 20-fold with 0.1% pepton water under constant shaking for 15 min at 160 rpm and at 10 °C. The serial decimal dilutions were prepared using pepton water, and they were spread-plated in triplicate onto Oxford Listeria Selective Agar (Merck, Darmstad, Germany) with Oxford Listeria Selective Supplement (Merck, Darmstad, Germany) and Violet Red Bile (VRB) Agar (Merck, Darmstad, Germany) for enumeration of *L. innocua* and *E. coli*, respectively. The plates were incubated at 37 °C for 24 h and the colonies were counted in triplicate plates. Microbiological counts were expressed as colony-forming unit per gram (CFU g^{-1}) of each film; the film without EOs was considered the control film.

2.2.4. Antimicrobial activity of films on inoculated melons

The antimicrobial activity of 2% EUG-containing films were also tested on inoculated melons. The melons were first washed using tap water, followed by ethanol (70%, w/w) and sterile distilled water. Six melons from each of the two different cultivar were left to dry under the laminar flow hood. Two separate zones (4 cm × 4 cm) on each melon's surface were then inoculated by spreading 200 µL of the two cultures (one zone with *L. innocua* and the other with *E. coli*). The inoculums of *L. innocua* and *E. coli* with 1×10^7 CFU/mL were prepared as described in Section 2.2.3. The inoculated melons were kept under aseptic conditions for 30 min to promote the absorption and drying of the inoculum on the melon surface. Freshly prepared solutions of zein containing EUG at 2% and control zein film solutions (500 µL) were then pipetted onto the inoculated areas (4 cm × 4 cm) of melons and spread homogeneously using a sterile plastic rod. The melons were kept under the laminar flow hood to allow solvent evaporation from the zein-based solutions on their surface. After 1 h (time 0), they were cold-stored at 10 °C and 50% RH for 7 days (time 7).

Microbiological tests were carried out at time 0 and time 7 by first unpeeling the coatings from the inoculated melon surface (4 cm × 4 cm area). The inoculated areas were then carefully cut with a sterile knife and separated from the fruits. Samples of ~10 g were then placed into a stomacher bag with 90 mL sterile 0.1% peptone water and homogenized (BagMixer ® 400, Interscience, France) for 120 s. The serial decimal dilutions prepared from this homogenate were then spread-plated (0.1 mL) in triplicate onto Oxford Listeria Selective Agar (Merck, Darmstad, Germany) with Oxford Listeria Selective Supplement (Merck, Darmstad, Germany) and Violet Red Bile (VRB) Agar (Merck, Darmstad, Germany) for enumeration of *L. innocua* and *E. coli*, respectively. The plates were incubated at 37 °C for 48 h and the colonies were counted in triplicate plates. Microbiological counts were expressed as colony-forming unit per gram (CFU g^{-1}) for each melon cultivar.

2.2.5. Tensile properties

Tensile strength, elongation at break, and Young's modulus of films were determined using a Texture Analyzer (TA-TX2, Stable Microsystems, Goldalming, UK) according to ASTM Standard Method D 882-02 (ASTM 2002). The films were cut into stripes 8 mm wide and 80 mm long before being tested. The initial grip distance was 50 mm, and the crosshead speed was 25 mm/min. At least seven measurements were conducted for each film, and the films' thickness was measured with a micrometer (Chronos®, UK).

2.2.6. Atomic Force Microscopy (AFM) measurements

AFM images of the surface of zein films were carried out by a Bruker MultiMode Nanoscope V in intermittent-contact mode in air with silicon tips (resonance frequency ≈340 kHz, spring constant ≈40 N/m, tip radius 8 nm). The captured images (3 or 4 for each sample) were analyzed by Nanoscope Analysis software v.1.7 (Bruker). The surface roughness R_{rms} was calculated as the root mean square average of height deviations (Z_i) taken from a mean data plane ($\bar{Z}$).

$$R_{rms} = \sqrt{\frac{\sum_{i=1}^{N}(Z_i - \bar{Z})^2}{N-1}}$$

The R_{max} parameter indicates the maximum vertical distance between the highest and the lowest points in the image. The particles analysis to determine the number, area, and depth of the holes set the threshold level at about 45 nm for the control, 1% EUG, and 2% EUG-containing film samples, but 8 nm for the 3% EUG-containing sample (this explains the very different values for the height minimum parameter).

2.2.7. Scanning Electron Microscopy (SEM) measurements

The SEM images of the films' cross-section were obtained using a 250 Quanta FEG (FEI Company, United States). The films were prepared by crushing and then freezing them in liquid nitrogen.

2.2.8. Surface wettability

Water contact angle measurements were performed on both the pristine control zein films and the EUG-containing zein films using an optical contact angle apparatus (OCA 15 Plus, Data Physics Instruments GmbH, Filderstadt, Germany) equipped with a high-resolution CCD camera and a high-performance digitizing adapter. SCA20 software (Data Physics Instruments GmbH, Filderstadt, Germany) was used for the image capturing and contact angle determination. Rectangular specimens (3 × 1.5 cm^2) were kept flat throughout the analysis, and the contact angle of water in air (θ, deg) was measured by gently dropping a droplet of 4.0 ± 0.5 mL of Milli-Q water (18.3 MV cm) onto the substrate at 23 ± 1 °C and 50 ± 2% relative humidity (RH). The contact angle was measured as soon as the droplet touched the film's surface (t_0) and during 30 s (t_{30}) as the angle between the baseline of the drop and the tangent at the drop boundary. These two time frames were arbitrarily selected to evaluate the contact angle evolution over time.

2.2.9. Oxygen transmission rate (OTR)

The oxygen barrier properties of the films were assessed using a Multiperm permeability analyzer (Extrasolution Srl, Capannori, Italy) equipped with an electrochemical sensor. Zein films were sandwiched between two aluminum-tape masks, allowing a surface of 2.5 cm^2 to be exposed to the permeation of oxygen. The oxygen transmission rate (OTR, $cm^3\,m^{-2}\,24\,h^{-1}$) was determined according to the standard method of ASTM D3985-10, with a carrier flow (N_2) of 10 mL min^{-1} at 23 °C and 0% relative humidity (RH), and at 1 atm pressure difference on the two sides of the specimen. Each OTR value was from three replicates.

2.2.10. Statistical analysis

Results were analyzed for significance by the Fisher's protected least significant difference method. Differences were considered significant if $P \leq 0.05$.

3. Results and discussions

3.1. Effect of the essential oils on the antimicrobial activity of films based on inhibition zones

The classical zone inhibition test is a semi-quantitative method that provides some basic information to estimate the potential success of antimicrobial films in food systems (Joerger, 2007). However, it is a scientific truth that forming a large zone in this test depends not only on the potency of the antimicrobial agent, but also on factors affecting its solubility (being in amorphous, crystalized, bound, or entrapped form) in the film, and its diffusion rates (from film to agar, and within the agar) (Ünalan et al., 2011; Yemenicioğlu, 2016; Boyacı & Yemenicioğlu, 2018). Thus, to minimize interference from differences in solubility and diffusion of EOs, the effect of both standard (24 h) and extended (48 h) incubation periods at 37 °C on zone areas were considered. The inhibition zones measured for the different EOs loaded into the zein films are reported in Table 1 for both *E. coli* and *L. innocua*. The minimum loading to observe clear zones was 2% for CAR and THY and 3% for EUG. These results suggest that the antimicrobial activity of CAR and THY-containing films against *E. coli* and *L. innocua* was higher than the

Table 1
Inhibition zones for the different EOs loaded into the zein films against *E. coli* and L. *innocua*.

Essential oil in film	Concentration (%) (w/w)	Zone area-24 h incubation (mm^2)		Zone area-48 h incubation (mm^2)	
		L. innocua	*E.coli*	*L. innocua*	*E.coli*
Zein control	–	nz^{F}	nz	nz	nz
Eugenol	0.5	nz	nz	nz	nz
	1	nz	nz	nz	nz
	2	nz	nz	nz	nz
	3	$58.44 \pm 10.1^{E,b}$	$24.23 \pm 6.3^{E,c}$	$71.17 \pm 13.9^{E,a}$	$52.97 \pm 6.4^{D,b}$
	4	$143.3 \pm 15.5^{B,a}$	$143.3 \pm 16.2^{B,a}$	$149.3 \pm 27.3^{C,a}$	$138.9 \pm 22.9^{B,a}$
	5	$165.3 \pm 17.6^{B,a}$	$152.0 \pm 15.2^{B,a}$	$162.8 \pm 16.3^{C,a}$	$155.4 \pm 24.5^{B,a}$
Carvacrol	0.5	nz	nz	nz	nz
	1	nz	nz	nz	nz
	2	$99.99 \pm 39.30^{D,ab}$	$110.69 \pm 30.44^{C,a}$	$93.37 \pm 39.90^{E,b}$	$106.54 \pm 30.69^{C,ab}$
	3	$195.29 \pm 29.69^{A,b}$	$218.61 \pm 20.19^{A,a}$	$205.98 \pm 34.70^{B,b}$	$202.47 \pm 20.79^{A,b}$
Thymol	0.5	nz	nz	nz	nz
	1	nz	nz	nz	nz
	2	$122.48 \pm 15.85^{C,a}$	$70.52 \pm 20.70^{D,b}$	$121.48 \pm 13.90^{D,a}$	$68.56 \pm 27.92^{D,b}$
	3	$154.21 \pm 20.14^{B,c}$	$227.1 \pm 45.84^{A,a}$	$230.64 \pm 24.47^{A.a}$	$212.08 \pm 42.98^{A,b}$

$^{A-E}$Values at each column followed by different capital letters indicate statistically significant differences ($P < 0.05$).
$^{a-c}$ Values at each row followed by different lower-case letters indicate statistically significant differences ($P < 0.05$). ^{F}nz: no zone.

EUG-loaded films. The extension of the incubation period from 24 to 48 h caused a significant increase in the zone area (1.5-fold) of 3% THY-containing films against *L. innocua*. In contrast, no change occurred in the zone areas of the films containing CAR against *L. innocua* after extending the incubation time. It seemed that the release of CAR from films ceased in the first 24 h of incubation at 37 °C, while 3% THY-containing films continued their release between 24 h and 48 h of incubation. Therefore, comparison of the results obtained from the THY and CAR-containing films must be made with care. However, the zones obtained for 2% THY (after 24 h and 48 h incubations) and 3% THY-containing films (after 48 h incubation) against *L. innocua* were significantly larger than the films containing 2% and 3% CAR, respectively. Thus, it could be concluded that the THY-containing films showed the highest antilisterial capacity. On the other hand, 2% CAR-containing films caused significantly larger zones on *E. coli* than 2% THY-containing films, while no significant differences were observed between the antimicrobial activity of 3% THY and 3% CAR-containing films against *E. coli*. A significant increase in the zone areas (2-fold) after the extension of the test period was also observed on *E. coli* with 3% EUG-containing films. However, the antimicrobial performance of the 3% EUG-containing films after 48 h was comparable only with the 2% THY-containing films. In the literature, there are different reports related to the antimicrobial performance of EUG, CAR, and THY. García-García, López-Malo, and Palou, (2011) reported that the antilisterial capacity for EOs (based on the minimum bactericidal concentration in broth medium) is CAR > THY > EUG. Gochev and Girova (2009), who conducted antimicrobial tests with broth media and with agar diffusion tests, reported EUG, CAR, and THY's similar antimicrobial potential against *E. coli*. On the other hand, Miladi et al. (2017) investigated the antimicrobial potency of EOs against twelve strains of *Salmonella Typhimirium* and reported the order of their effectiveness as THY > CAR > EUG. It is also important to note that Alkan & Yemenicioğlu (2016) reported that zein coatings with EOs such as EUG, CAR, THY, and citral were effective on the main bacterial plant pathogens, such as *Erwinia amylovora, Ervinia caratovora,* and *Xanthomonas vesicatoria*. Moreover, it was also determined that the EUG-containing films were the only effective films against *Pseudomonas syringae* (Alkan & Yemenicioğlu, 2016).

3.2. Effect of the essential oils on the antimicrobial activity of films based on surface inoculation

The results of the film surface inoculation test are displayed in Table 2. The *E. coli* counts of all EOs-containing films were less than 2 Log CFU/g at time 0, 24 h, and after 7 days at 10 °C. Therefore, *E. coli* showed minimum 2.7 Decimal (D) reduction upon contact with the surface of films loaded with 2% of the three EOs. The *E. coli* inoculated on the control film showed ˜1.6 D reduction after 24 h and > 2.7 D reduction and after 7 days. This suggests that the hydrophobic surface of the zein film is not a suitable medium for the survival and growth of *E. coli*. *L. innocua* showed a greater resistance to EOs-containing films than *E. coli*; while no significant reduction was observed in *L. innocua* counts for the 2% EUG-containing films at time 0 ($P > 0.05$), ˜2 D and 1.6 D reductions were observed in *L. innocua* counts of 2% CAR and 2% THY-containing films at time 0, respectively ($P < 0.05$). *L. innocua* counts of all EOs-containing films dropped below 2 Log CFU/g (minimum 3.9 D reduction) after 24 h and 7 days. The same behavior was observed for the control films (˜3.3 D reduction after 24 h and ≥ 3.9 D reduction after 7 days). These results clearly showed the effectiveness of EOs in reducing the proliferation of *E. coli* and *L. innocua* on the zein films' surface. Moreover, although zein does not have inherent antimicrobial activity (Mecitoğlu et al., 2006; Ünalan et al., 2013; Arcan & Yemenicioğlu, 2013), the low water activity of dry hydrophobic zein films might be a factor that causes bacterial inactivation on

Table 2
Film surface inoculation-based antimicrobial activity of zein films containing essential oil.

Essential oil in film	Concentration (%) (w/w)	Storage time (days)	*L. innocua* (Log CFU/g)	*E. coli* (Log CFU/g)
Zein control	–	0	$5.90 \pm 0.16^{A,a}$	$4.72 \pm 0.25^{A,b}$
EUG	2	0	5.29 ± 0.73^{A}	< 2
CAR	2	0	3.82 ± 0.73^{B}	< 2
THY	2	0	4.31 ± 0.83^{B}	< 2
Zein control	–	1	$2.64 \pm 0.26^{C,a}$	$3.15 \pm 0.21^{B,a}$
EUG	2	1	< 2	< 2
CAR	2	1	< 2	< 2
THY	2	1	< 2	< 2
Zein control	–	7	< 2	< 2
EUG	2	7	< 2	< 2
CAR	2	7	< 2	< 2
THY	2	7	< 2	< 2

$^{A–B}$ Values at each column followed by different capital letters indicate statistically significant differences ($P < 0.05$).
$^{A–B}$Values at each row followed by different lower-case letters indicate statistically significant differences ($P < 0.05$).

the surface of the control films.

3.3. Antimicrobial activity of selected coatings on the melon surface

The antimicrobial tests in the laboratory medium showed that the minimum inhibitory concentrations of EOs in the films were considerably high (2–3%). Although, CAR and THY are more potent antimicrobials than EUG they are classified as food additives by the US Food and Drug Administration (FDA) (21 CFR, Part 172, 2017). On the other hand, EUG has been defined as a generally recognized as safe (GRAS) substance by the FDA (21 CFR, Part 184, 2018). For this reason, this work incorporated EUG in the zein coatings tested on inoculated melon peels. The results of *L. innocua* and *E. coli* counts at time 0 and after 7 days of cold storage at 10 °C for the control (uncoated melons), melons coated with zein, and melons coated with 2% EUG-containing zein are shown in Table 3. First of all, it should be reported that the *L. innocua* and *E. coli* counts of two different melon cultivars, Crenshaw (Cultivar #1) and Santa Claus (Cultivar #2), were quite similar. The *L. innocua* and *E. coli* inoculated at the surface of melons showed a high stability and changed only by ≤ 0.33 and ≤ 0.2 decimals (D) after 7-day cold storage, respectively. At time 0 (cold storage), inoculated melons coated with zein film showed 1.68–2.30 decimal (D) lower *L. innocua* (Average D reduction: -1.81 D) and *E. coli* (Average D reduction: -2.09 D) counts than inoculated control melons. The antimicrobial effect of ethanol on *Listeria* spp. and *E. coli* is well known (Kapetanakou, Karyotis, & Skandamis, 2016). Thus, this result suggests that the ethanol present in the zein film solutions applied on the melon surfaces might have played a role in the overall antimicrobial effect of the films. Cold storage at 10 °C for 7 days caused only 0.08 to 0.63 D increase in the number of inoculated pathogens on the melon surfaces coated with the zein films; however, *L. innocua* and *E. coli* counts of inoculated melons coated with zein films at the end of 7 days were still 1–1.9 D lower than those of the control samples. The difference in terms of decimal reductions obtained at time 0 in pathogenic counts of melons coated with zein films and 2% EUG-containing zein films were very limited (from 0.02 D to 0.8 D). In contrast, the *L. innocua* and *E. coli* counts of melons coated with 2% EUG-containing zein films were respectively almost 2 D and 1 D lower than those of zein-film-coated melons at the end of 7 days. These results suggest that the reduction in microbial counts at time 0 was due mainly to the antimicrobial effect of ethanol retained in the coatings for several hours before evaporation. At the same, it appeared that the antimicrobial reduction that occurred after 7 days was due to the EUG released from the films. It is important to note that the total decimal difference between *L. innocua* and *E. coli* counts of uncoated inoculated melons and melons coated with 2% EUG-containing zein films reached almost 3D (average D reductions were -3.28 and -2.94, respectively). These results represent clear evidence of the developed coatings' effectiveness with inactivating pathogenic indicators tested on the melon surfaces.

3.4. Effect of the essential oils on the films' mechanical properties

The structure of zein films is a meshwork of doughnut structures joined together by asymmetric rods (Guo, Liu, An, Li, & Hu, 2005). It is the hydrophobic interactions in particular that keep the zein rods together and maintain the film's integrity. However, these interactions are also responsible for the brittleness and lack of flexibility in zein films. The effects of different EOs on tensile strength, elongation at break, and Young's modulus in these films are summarized in Table 4. EOs had different plasticizing capacities on zein films depending on their concentrations. The use of EUG at 0.5% did not cause a significant change in the elongation at break of films ($P > 0.05$). However, EUG had the best plasticizing effect on zein films, as demonstrated by the 297% elongation at break for the 1% EUG-containing film samples. The elongation-at-break values of zein films increased at a concentration-dependent manner as EUG concentration of films increased gradually from 1% to 3%. The use of EUG at 0.5% caused a slight but significant ($P < 0.05$) increase in tensile strength of films, while it caused a significant reduction in the Young's modulus. In contrast, an increase of EUG from 1% to 3% caused a concentration-dependent reduction in both tensile strength and Young's modulus.

The THY acted as a less effective plasticizer for the zein films compared to EUG, with a considerable increase in the elongation at break only at the concentration of 2%. Moreover, further increase in THY from 2% to 3% did not cause a significant change in the films' mechanical properties. This result clearly showed the limited plasticizing effect of THY. Since phenolic-film matrix interactions are known to cause the plasticization of zein films (Arcan & Yemenicioğlu, 2011), the antiplasticizing effect caused by THY is most likely due to phenolic-phenolic interactions among THY molecules, leading eventually to phenolic polymerization reactions.

The use of CAR at 0.5% or 1% caused a slight increase in the elongation of films. However, increasing the CAR loading to 3% yielded a considerably higher elongation at break of 119%, which supports the considerable plasticizing effect for the zein films. However, it should be pointed out that EUG at 1% and 2% was a much more effective plasticizer than CAR at the same concentrations. CAR at 0.5% and 1% led to a significant ($P < 0.05$) increase of the tensile strength of the films, while CAR at 2% gave tensile strength values of the same order of magnitude as those recorded for the control. In contrast, CAR at 3% caused a significant reduction in the films' tensile strength. The use of CAR reduced the Young's modulus of films regardless of the

Table 3
Changes in *L. innocua* and *E. coli* counts on inoculated surfaces of whole melons during cold storage at 10 °C.

Coating type	Time (days)	Cultivar #1	Cultivar #2	Average of decimal changes[c]
		L. innocua (Log CFU/g)		
Uncoated	0	4.59 ± 0.21 (0.00)[a]	4.66 ± 0.17 (0.00)	0.00
Zein	0	2.65 ± 0.44 (-1.94)	2.98 ± 0.06 (-1.68)	−1.81
Zein-2% EUG	0	2.23 ± 0.18 (-2.36)	2.25 ± 0.18 (-2.41)	−2.39
Uncoated	7	4.41 ± 0.24 (-0.18)	4.33 ± 0.30 (-0.33)	−0.26
Zein	7	3.28 ± 0.13 (-1.31)	3.36 ± 0.17 (-1.30)	−1.31
Zein-2% EUG	7	1.30 ± 0.25 (-3.29)	1.39 ± 0.27 (-3.27)	−3.28
		E. coli (Log CFU/g)		
Uncoated	0	4.47 ± 0.12 (0.00) [b]	4.04 ± 0.03 (0.00)	0.00
Zein	0	2.17 ± 0.60 (-2.30)	2.17 ± 0.56 (-1.87)	−2.09
Zein-2% EUG	0	2.19 ± 0.26 (-2.28)	2.10 ± 0.11 (-1.94)	−2.11
Uncoated	7	4.27 ± 0.03 (-0.20)	4.16 ± 0.13 (+0.12)	−0.04
Zein	7	2.56 ± 0.19 (-1.91)	2.25 ± 0.26 (-1.79)	−1.81
Zein-2% EUG	7	1.35 ± 0.15 (-3.12)	1.20 ± 0.28 (-2.84)	−2.94

[a,b]For each cultivar, values in parentheses report decimal changes in samples' *L. innocua* or *E. coli* counts compared to that of the uncoated control at the 0th day.
[c]Average of decimal changes for cultivar #1 and cultivar #2.

Table 4
Effects of different essential oils on mechanical properties of zein films.

Essential oil in film	Concentration (%) (w/w)	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)
Zein control	–	12.71 ± 3.14 ^{D}	0.82 ± 0.19 ^{H}	257.45 ± 68.69 ^{A}
Carvacrol	0.5	29.53 ± 6.06 ^{A}	35.03 ± 9.96 ^{FG}	181.54 ± 27.98 ^{BC}
	1	25.39 ± 1.96 ^{B}	26.90 ± 8.95 ^{FG}	171.98 ± 10.66 ^{C}
	2	15.39 ± 3.40 ^{CD}	118.70 ± 21.33 ^{E}	106.48 ± 21.10 ^{D}
	3	4.67 ± 0.54 ^{EF}	423.92 ± 29.48 ^{B}	10.33 ± 2.17 ^{F}
Thymol	0.5	0.69 ± 0.22 ^{FG}	1.76 ± 0.57 ^{H}	2.49 ± 0.23 ^{F}
	1	2.51 ± 1.37 ^{FG}	2.56 ± 0.52 ^{H}	1.63 ± 0.14 ^{F}
	2	3.99 ± 0.78 ^{EF}	39.76 ± 5.36 ^{FG}	1.61 ± 0.26 ^{F}
	3	3.38 ± 0.16 ^{EFG}	48.77 ± 12.57 ^{F}	1.30 ± 0.24 ^{F}
Eugenol	0.5	28.61 ± 4.18 ^{A}	15.92 ± 5.23 ^{GH}	204.95 ± 25.92 ^{B}
	1	17.26 ± 3.65 ^{C}	296.99 ± 47.12^{D}	64.08 ± 15.03 ^{F}
	2	6.16 ± 1.68 ^{E}	386.02 ± 34.36^{C}	6.54 ± 1.34 ^{F}
	3	2.76 ± 0.49 ^{EFG}	472.81 ± 36.90^{A}	1.65 ± 0.58 ^{G}

$^{A-H}$ Values at each column followed by different capital letters indicate statistically significant differences ($P < 0.05$).

concentration used, but films containing CAR showed higher Young's modulus values than zein films loaded with the other EOs at the same concentration.

Overall, EUG was the best plasticizer for zein films, in line with previous findings by Alkan & Yemenicioğlu (2016), who identified EUG as the most effective plasticizer of zein among different tested phenolic acids, essential oils, and phenolic extracts. It is thought that the H-bonding between hydroxyl groups of phenolic compounds and peptide carbonyl groups of zein has a role in the observed plasticizing effect (Arcan & Yemenicioğlu, 2011). It appears that the binding of phenolic compounds onto tightly joined asymmetric zein rods caused an increase in the zein film matrix's free volume. The hydroxyl groups of phenolic compounds within the film matrix also reduced the hydrophobicity of the zein film, which might mitigate the films' problems of brittleness and flexibility (Alkan et al., 2011; Arcan & Yemenicioğlu, 2011, 2013).

Because of the promising results obtained for the EUG-loaded films (in terms of antimicrobial performance on inoculated melons and mechanical properties), further characterization studies were focused on these films, keeping the bare zein films as the control.

3.5. Morphological characterization

The surface morphology of zein films with and without EUG was evaluated by AFM, highlighting topographical features at a nanometer scale. The most relevant parameters gathered from the AFM analysis are reported in Table 5, whereas two representative images (control and 3% EUG-containing film samples) are displayed in Fig. 1. The surface morphology of zein films (both with and without EUG) was apparently made of two distinct phases, more pronounced in the sample that did not contain EUG. This suggests a phase separation between zein, the main film polymer and glycerol, the plasticizer. The addition of EUG up to 2% led to a significant decrease in the hole number, while a further increase in EUG to 3% again caused an increase in the hole number close to that of the bare zein films (Table 5). On the other hand, it is interesting to note that for the highest amount of EUG (at 3%), the number of small holes (area < 0.1 μm^2) in the zein film was dramatically higher than those of the control and 1% and 2% EUG-containing zein films (Fig. 2). However, the range of larger particles (area between 0.1 and 0.5 μm^2) was higher for the control films than the EUG-containing films that showed almost similar range of these larger particles. Finally, the quantity of largest particles (area between 0.5 and 1.0 μm^2) found in all the samples was close to each other. The high number of small holes in the sample containing the highest amount of EUG (at 3%) suggests an emulsifying effect originating from the essential oil, which somehow acted as a compatibilizer between zein and glycerol. This is corroborated by both the 3% EUG-containing film sample's roughness values (R_{rms} and R_{max}) which are ˜20 and ˜7 times lower than those of the other film samples (control, 1% EUG and 2% EUG-containing films). In a similar way, the lowest height values (H_{min} and H_{max}) were measured for the EUG-richest film samples (EUG at 3%), and this once again denotes the homogenizing effect of the essential oil's highest concentration (Table 5).

The SEM micrographs of the films' cross-section (Fig. 3) are in line with the AFM findings, clearly showing that the control zein films are highly porous. The incorporation of EUG into films between 0.5% and 1% did not bring any apparent change in film morphology. In contrast, in the presence of EUG at 3%, the film morphology appeared denser compared to the other samples, and it showed very small holes rather than large domains. Thus, it seemed that at the highest EUG concentration the affinity between zein and EUG, driven by extensive phenolic-phenolic interactions, increased the film networking and thereby reduced the film porosity. The increased porosity of zein films by incorporation of different phenolic compounds was reported previously by Arcan and Yemenicioğlu (2011). However, concentration-dependent changes in film morphology (moving from porous to dense by increasing the loading of the essential oil) was first reported in this study.

3.6. Surface wettability

The optical contact angle analysis is a powerful technique that allows the researcher to gather thermodynamic information from a geometrical measurement, e.g. the angle at the interface between the solid, liquid, and gas phases (Karbowiak, Debeaufort, & Voilley, 2006; Karbowiak, Debeaufort, Champion, & Voilley, 2006). In the food

Table 5
Main morphological parameters drawn from the AFM analysis of the surface of different zein films.

Sample	Holes number	R_{rms} (nm)	R_{max} (nm)	$Area_{min}$ (μm^2)	$Area_{max}$ (μm^2)	H_{min} (nm)	H_{max} (nm)
Control	149 ± 20^{A}	150 ± 31^{A}	1309 ± 167^{A}	0.010 ± 0.001^{A}	1.618 ± 1.025^{A}	51 ± 6^{A}	820 ± 191^{A}
EUG 1%	71 ± 10^{B}	179 ± 19^{A}	1094 ± 83^{A}	0.012 ± 0.003^{A}	1.669 ± 0.646^{A}	46 ± 8^{A}	828 ± 99^{A}
EUG 2%	40 ± 12^{C}	197 ± 25^{A}	1082 ± 248^{A}	0.017 ± 0.011^{A}	4.382 ± 0.548^{B}	64 ± 6^{A}	940 ± 145^{A}
EUG 3%	135 ± 34^{A}	9 ± 1^{B}	167 ± 89^{B}	0.010 ± 0.000^{A}	1.873 ± 0.200^{A}	9 ± 1^{B}	116 ± 69^{B}

Different letters denote a statistically significant difference between samples within each group ($P < 0.05$).

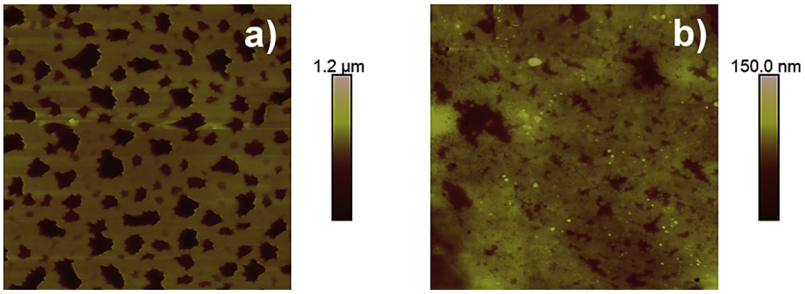

Fig. 1. Atomic force microscopy height images (10 × 10 μm^2) of pristine zein film (control, a) and zein film loaded with 3% eugenol (EUG 3%, b). In detail, for the control (panel a): R_{rms} = 173 nm; R_{max} = 1.22 µm; holes area: 27%. For the EUG 3% (panel b): R_{rms} = 10 nm; R_{max} = 230 nm; holes area: 14%.

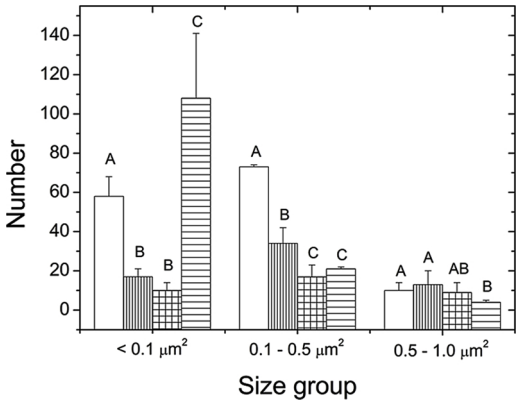

Fig. 2. Number of particles within three main size groups visualized by AFM on the four zein film formulations tested in this work (empty pattern: control; vertical line pattern: EUG 1%; webbed pattern: EUG 2%; horizontal line pattern: EUG 3%). Different letters denote a statistically significant difference between samples within each size group ($P < 0.05$).

packaging sector, the use of the contact angle technique especially pertains to the characterization of plastics. More recently, this approach has been extended to biopolymers (Karbowiak, Debeaufort, Voilley et al., 2006; Karbowiak, Debeaufort, Champion et al., 2006) in the form of coatings and edible films. The goal is to obtain information on wettability properties toward a specific liquid in order to predict sorption phenomena, or to control the formation of biofilms.

Because of the inherent hydrophilic nature of biopolymers, attaining an equilibrium water contact angle (which is at the root of Young's theory) is extremely complicated by phenomena such as absorption, adsorption, spreading, and evaporation (Farris et al., 2011). For this reason, it is often more expedient to monitor the evolution of the contact angle over a specific temporal window rather than focusing on static values. In this study, the wettability of the zein films had been investigated during 30 s (Fig. 4). The overall trend is the same for the four samples investigated (control, 1% EUG, 2% EUG, and 3% EUG-containing films), with a rapid decrease of the contact angle during the first seconds upon the water droplet contacting the film surface. This behavior is typical of biopolymers and can be explained by the combined effect of both spreading and absorption on the film surface (Farris et al., 2011). However, the initial contact angles were significantly different among samples: 35.85 ± 5.1, 36.04 ± 3.8, 42.56 ± 2.3, and 63.38 ± 1.7 for the control, 1% EUG, 2% EUG, and 3% EUG-containing film samples, respectively. These values seem to follow the increase in the concentration of EUG. However, the sudden decrease of θ (°) suggests that the hydrophilic groups of the film matrix orient to minimize interfacial energy at the solid-liquid interface. After 30 s, a

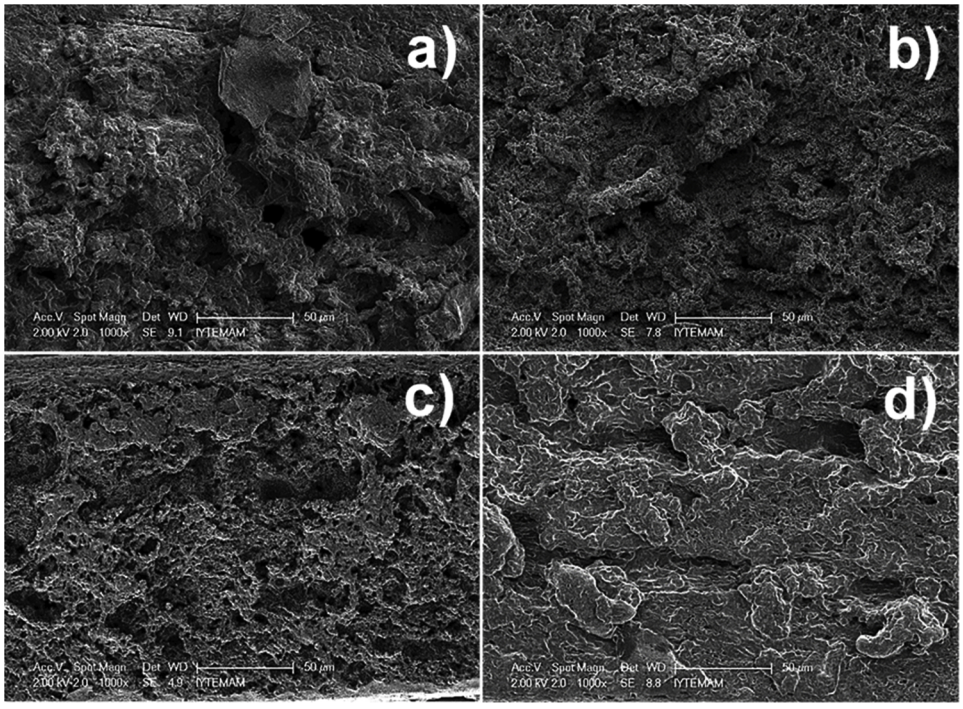

Fig. 3. Effect of EUG on the morphology of zein films: a) control; b) 0.5% EUG; c) 1% EUG; d) 3% EUG.

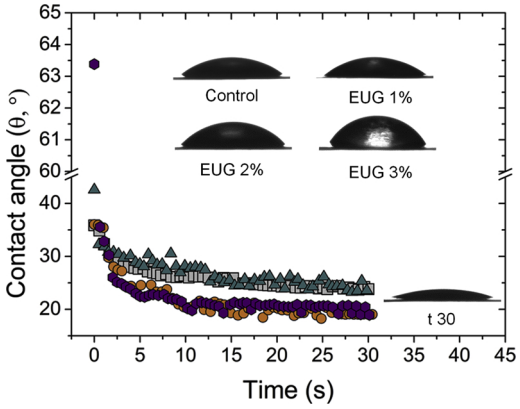

Fig. 4. Water contact evolution recorded on the surface of the zein films (control, gray square) and the samples EUG 1% (orange circle), EUG 2% (dark cyan triangle), and EUG 3% (purple hexagon). Some representative images for the four samples recorded at time 0 (i.e., as the water droplet touched the sample surface) are shown at the upper part of the figure. A representative image of the water droplet after 30 s evolution is shown at the lower-right side of the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

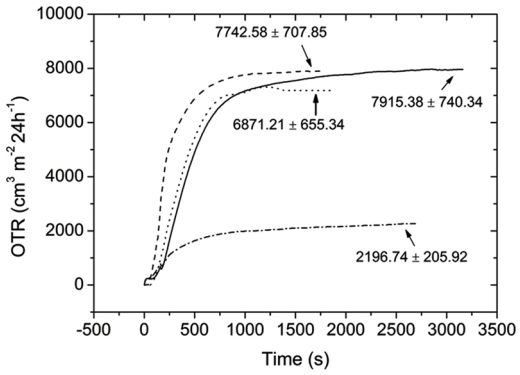

Fig. 5. Representative curves of the OTR evolution recorded for the zein films (control, solid line) and the samples EUG 1% (dashed line), EUG 2% (dotted line), and EUG 3% (dashed-dotted line). The OTR values close to each curve are the average OTR (± standard deviation) gathered from three replicates. The different durations of the analysis indicates a different time necessary to achieve a steady state condition for each sample type.

pseudo-equilibrium was apparently achieved for the four samples at θ between 19° and 23°.

3.7. Oxygen transmission rate

The gas and vapor permeability properties of edible films and coatings are crucial to tailor the material's performance for the final application; this permeability (especially of O_2 and CO_2) is of particular interest for respiring fresh fruits and vegetables in order to evaluate the coating's impact on the food's metabolism. In contrast, a high barrier performance against oxygen could be sought for oxygen-sensitive foods, such as those rich in fats.

In this work, the oxygen-barrier properties of zein films made with and without EUG were tested. The oxygen transmission rate evolution and the final mean value (OTR, $cm^3\ m^{-2}\ 24\ h^{-1}$ at 23 °C and 0% RH) for the different samples are reported in Fig. 5. The addition of EUG up to 2% did not have any significant impact on the oxygen barrier performance compared to the control film. At 3% EUG loading, however, the OTR dropped significantly from ˜ 7700 $cm^3\ m^{-2}\ 24\ h^{-1}$ to 2200 $cm^3\ m^{-2}\ 24\ h^{-1}$. This observation is somehow opposite from the established effect of EOs on the oxygen permeability of films and coatings described in previous works (Ejaz, Arfat, Mulla, & Ahmed, 2018; Han, Yu, & Wang, 2018). Accordingly, a depletion of the oxygen barrier performance is expected with increasing the EO concentration, mainly due to less cohesive forces of apolar matrices compared with the bare biopolymer (where extensive intra- and intermolecular hydrogen bonding prevail), and the higher solubility of the permeant (i.e., oxygen) in hydrophobic matrices.

To explain the observed trend, one should remind the effect of the highest concentration of EUG on the zein-based films' morphology. At 3% EUG loading, the surface and bulk appearance of the films appeared regular and smoothed compared to the irregular, holed, and rough morphology of the pristine zein film, as shown by the AFM and SEM analyses. In other words, the homogenizing effect of EUG at the highest concentration (3%) might also have had an effect on the oxygen barrier properties, limiting the detrimental consequences from the presence of discontinuities throughout the main biopolymer phase as seen in the pristine zein films. At the same time, it is clear that none of the formulations tested yielded a true oxygen barrier coating, as demonstrated by the high OTR values. In fact, the barrier properties of EUG-containing films were similar to those of oil-based polymers such as low-density polyethylene (LDPE 40 μm) and polypropylene (PP, 30 μm), which are well known to be poor oxygen barrier materials. Nevertheless, the OTR values recorded for the 3% EUG-containing zein films could be suitable for the intended application, because the deposition of the coating could slow down the respiration rate of the fruit (thereby delaying the senescence process) without completely blocking the oxygen transfer between the surrounding environment and the food.

4. Conclusions

This investigation showed the possibility of obtaining flexible antimicrobial films of zein incorporated with essential oils. It was also showed that the developed flexible films could be employed as antimicrobial coatings for the inhibition of some major pathogenic bacteria on the peel surfaces of whole melons. Thus, the coatings developed in this work might serve not only as a solution to control worldwide human infections originated from melons, but also to tackle the economic losses caused by microbial spoilage of whole melons. Further studies are needed to combine essential oils with suitable flavor compounds and to suppress the coatings' undesired odors from the essential oils. Moreover, optimization of the coating's thickness is needed for commercially important melon cultivars that differ in their peel characteristics (smooth, rough or webbed), respiratory profiles, and physiological status.

Acknowledgements

We thank the Material Research Center (IYTE MAM) of the Izmir Institute of Technology for conducting the films' SEM analysis. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Alkan, D., & Yemenicioğlu, A. (2016). Potential application of natural phenolic antimicrobials and edible film technology against bacterial plant pathogens. *Food Hydrocolloids, 55*, 1–10. https://doi.org/10.1016/j.foodhyd.2015.10.025.

Alkan, D., Aydemir, L. Y., Arcan, I., Yavuzdurmaz, H., Atabay, H. I., Ceylan, C., et al. (2011). Development of flexible antimicrobial packaging materials against Campylobacter jejuni by incorporation of gallic acid into zein-based films. *Journal of Agricultural and Food Chemistry, 59*(20), 11003–11010. https://doi.org/10.1021/jf202584b.

Álvarez-Ordóñez, A., Leong, D., Hickey, B., Beaufort, A., & Jordan, K. (2015). The challenge of challenge testing to monitor Listeria monocytogenes growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document. *Food Research International, 75*, 233–243. https://doi.org/10.1016/j.foodres.2015.06.004.

And, M. A., & Yousef, A. E. (2001). Efficacy of ozone against Escherichia coli O157: H7 on apples. *Journal of Food Science, 66*(9), 1380–1384. https://doi.org/10.1111/j.1365-

2621.2001.tb15218.x.
Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies, 3*(2), 113–126. https://doi.org/10.1016/S1466-8564(02)00012-7.
Arcan, I., & Yemenicioğlu, A. (2011). Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. *Food Research International, 44*(2), 550–556. https://doi.org/10.1016/j.foodres.2010.11.034.
Arcan, I., & Yemenicioğlu, A. (2013). Development of flexible zein–wax composite and zein–fatty acid blend films for controlled release of lysozyme. *Food Research International, 51*(1), 208–216. https://doi.org/10.1016/j.foodres.2012.12.011.
Assis, O. B. G., & Leoni, A. M. (2009). Protein hydrophobic dressing on seeds aiming at the delay of undesirable germination. *Scientia Agricola, 66*(1), 123–126. https://doi.org/10.1590/S0103-90162009000100017.
Bai, J., Alleyne, V., Hagenmaier, R. D., Mattheis, J. P., & Baldwin, E. A. (2003). Formulation of zein coatings for apples (Malus domestica Borkh) 1. *Postharvest Biology and Technology, 28*(2), 259–268. https://doi.org/10.1016/S0925-5214(02)00182-5.
Boyacı, D., & Yemenicioğlu, A. (2018). Expanding horizons of active packaging: Design of consumer-controlled release systems helps risk management of susceptible individuals. *Food Hydrocolloids, 79*, 291–300. https://doi.org/10.1016/j.foodhyd-.2017.12.038.
Centers for Disease Control and Prevention (2012). *Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen farms, Colorado.* Accessed 4 October 2018 https://www.cdc.gov/listeria/outbreaks/-cantaloupes-jensen-farms/index.html.
Chen, W., Jin, T. Z., Gurtler, J. B., Geveke, D. J., & Fan, X. (2012). Inactivation of Salmonella on whole cantaloupe by application of an antimicrobial coating containing chitosan and allyl isothiocyanate. *International Journal of Food Microbiology, 155*(3), 165–170. https://doi.org/10.1016/j.ijfoodmicro.2012.02.001.
Ejaz, M., Arfat, Y. A., Mulla, M., & Ahmed, J. (2018). Zinc oxide nanorods/clove essential oil incorporated Type B gelatin composite films and its applicability for shrimp packaging. *Food Packaging and Shelf Life, 15*, 113–121. https://doi.org/10.1016/j.fpsl.2017.12.004.
Farris, S., Introzzi, L., Biagioni, P., Holz, T., Schiraldi, A., & Piergiovanni, L. (2011). Wetting of biopolymer coatings: Contact angle kinetics and image analysis investigation. *Langmuir, 27*(12), 7563–7574. https://doi.org/10.1021/la2017006.
García-García, R., López-Malo, A., & Palou, E. (2011). Bactericidal action of binary and ternary mixtures of carvacrol, thymol, and eugenol against Listeria innocua. *Journal of Food Science, 76*(2), M95–M100. https://doi.org/10.1111/j.1750-3841.2010.02005.x.
Gennadios, A., Hanna, M. A., & Kurth, L. B. (1997). Application of edible coatings on meats, poultry and seafoods: A review. *LWT-Food Science and Technology, 30*(4), 337–350. https://doi.org/10.1006/fstl.1996.0202.
Ghanbarzadeh, B., Oromiehie, A. R., Musavi, M., D-Jomeh, Z. E., Rad, E. R., & Milani, J. (2006). Effect of plasticizing sugars on rheological and thermal properties of zein resins and mechanical properties of zein films. *Food Research International, 39*(8), 882–890. https://doi.org/10.1016/j.-foodres.2006.05.011.
Gochev, V. K., & Girova, T. D. (2009). Antimicrobial activity of various essential oils against spoilage and pathogenic microorganisms isolated from meat products. *Biotechnology, Biotechnological Equipment, 23*(sup. 1), 900–904. https://doi.org/10.1080/13102818.2009.10818568.
Gol, N. B., & Rao, T. R. (2014). Influence of zein and gelatin coatings on the postharvest quality and shelf life extension of mango (Mangifera indica L.). *Fruits, 69*(2), 101–115. https://doi.org/10.1051/fruits/2014002.
Guo, Y., Liu, Z., An, H., Li, M., & Hu, J. (2005). Nano-structure and properties of maize zein studied by atomic force microscopy. *Journal of Cereal Science, 41*(3), 277–281. https://doi.org/10.1016/j.jcs.2004.12.005.
Han, J. H. (2013). Antimicrobial packaging systems. In S. Ebnesajjad (Ed.). *Plastic films in food packaging* (pp. 151–180). Elsevier. https://doi.org/10.1016/B978-1-4557-3112-1.00010-7.
Han, Y., Yu, M., & Wang, L. (2018). Preparation and characterization of antioxidant soy protein isolate films incorporating licorice residue extract. *Food Hydrocolloids, 75*, 13–21. https://doi.org/10.1016/j.foodhyd.2017.09.020.
Hoa, T. T., Ducamp, M. N., Lebrun, M., & Baldwin, E. A. (2002). Effect of different coating treatments on the quality of mango fruit. *Journal of Food Quality, 25*(6), 471–486. https://doi.org/10.1111/j.1745-4557.2002.tb01041.x.
Izumi, H. (1999). Electrolyzed water as a disinfectant for fresh-cut vegetables. *Journal of Food Science, 64*(3), 536–539. https://doi.org/10.1111/j.1365-2621.1999.tb15079.x.
Janes, M. E., Kooshesh, S., & Johnson, M. G. (2002). Control of Listeria monocytogenes on the surface of refrigerated, ready-to-eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. *Journal of Food Science, 67*(7), 2754–2757. https://doi.org/10.1111/j.1365-2621.2002.tb08810.x.
Joerger, R. D. (2007). Antimicrobial films for food applications: A quantitative analysis of their effectiveness. *Packaging Technology and Science: An International Journal, 20*(4), 231–273. https://doi.org/10.1002/pts.774.
Kapetanakou, A. E., Karyotis, D., & Skandamis, P. N. (2016). Control of Listeria monocytogenes by applying ethanol-based antimicrobial edible films on ham slices and microwave-reheated frankfurters. *Food Microbiology, 54*, 80–90. https://doi.org/10.1016/j.fm.2015.10.013.
Karbowiak, T., Debeaufort, F., Champion, D., & Voilley, A. (2006). Wetting properties at the surface of iota-carrageenan-based edible films. *Journal of Colloid and Interface Science, 294*(2), 400–410. https://doi.org/10.1016/j.jcis.2005.07.030.
Karbowiak, T., Debeaufort, F., & Voilley, A. (2006). Importance of surface tension characterization for food, pharmaceutical and packaging products: A review. *Critical Reviews in Food Science and Nutrition, 46*(5), 391–407. https://doi.org/10.1080/10408390591000884.
Kim, S., Sessa, D. J., & Lawton, J. W. (2004). Characterization of zein modified with a mild cross-linking agent. *Industrial Crops and Products, 20*(3), 291–300. https://doi.org/10.1016/j.indcrop.-2003.10.013.
Lai, H. M., & Padua, G. W. (1997). Properties and microstructure of plasticized zein films. *Cereal Chemistry, 74*(6), 771–775. https://doi.org/10.1094/CCHEM.1997.74.6.771.
Lawton, J. W. (2004). Plasticizers for zein: Their effect on tensile properties and water absorption of zein films. *Cereal Chemistry, 81*(1), 1–5. https://doi.org/10.1094/CCHEM.2004.81.1.1.
Lin, L. S., Wang, B. J., & Weng, Y. M. (2011). Quality preservation of commercial fish balls with antimicrobial zein coatings. *Journal of Food Quality, 34*(2), 81–87. https://doi.org/10.1111/j.1745-4557.2011.00370.x.
Lungu, B., & Johnson, M. G. (2005). Fate of Listeria monocytogenes inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate, and sodium lactate at 4 C. *Journal of Food Protection, 68*(4), 855–859. https://doi.org/10.4315/0362-028X-68.4.855.
Ma, Q., Zhang, Y., Critzer, F., Davidson, P. M., & Zhong, Q. (2016). Quality attributes and microbial survival on whole cantaloupes with antimicrobial coatings containing chitosan, lauric arginate, cinnamon oil and ethylenediaminetetraacetic acid. *International Journal of Food Microbiology, 235*, 103–108. https://doi.org/10.1016/j.ijfoodmicro.2016.07.030.
Manley, R. H., & Evans, C. D. (1943). Binary solvents for zein. *Industrial and Engineering Chemistry, 35*(6), 661–665. https://doi.org/10.1021/ie50402a008.
Marcos, B., Aymerich, T., Monfort, J. M., & Garriga, M. (2007). Use of antimicrobial biodegradable packaging to control Listeria monocytogenes during storage of cooked ham. *International Journal of Food Microbiology, 120*(1-2), 152–158. https://doi.org/10.1016/j.ijfoodmicro.2007.06.003.
Mecitoğlu, Ç., Yemenicioğlu, A., Arslanoğlu, A., Elmacı, Z. S., Korel, F., & Çetin, A. E. (2006). Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging. *Food Research International, 39*(1), 12–21. https://doi.org/10.1016/j.foodres.2005-.05.007.
Mehyar, G. F., El Assi, N. M., Alsmairat, N. G., & Holley, R. A. (2014). Effect of edible coatings on fruit maturity and fungal growth on Berhi dates. *International Journal of Food Science & Technology, 49*(11), 2409–2417. https://doi.org/10.1111/ijfs.12562.
Miladi, H., Zmantar, T., Kouidhi, B., Chaabouni, Y., Mahdouani, K., Bakhrouf, A., et al. (2017). Use of carvacrol, thymol, and eugenol for biofilm eradication and resistance modifying susceptibility of Salmonella enterica serovar Typhimurium strains to nalidixic acid. *Microbial Pathogenesis, 104*, 56–63. https://doi.org/10.1016/j.micpath.2017.01.012.
Ouattara, B., Simard, R. E., Piette, G., Bégin, A., & Holley, R. A. (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *International Journal of Food Microbiology, 62*(1-2), 139–148. https://doi.org/10.1016/S0168-1605(00)00407-4.
Padgett, T., Han, I. Y., & Dawson, P. L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection, 61*(10), 1330–1335. https://doi.org/10.4315/0362-028X-61.10.1330.
Park, H. J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science & Technology, 10*(8), 254–260. https://doi.org/10.1016/S0924-2244(00)00003-0.
Park, H. J., Chinnan, M. S., & Shewfelt, R. L. (1994). Edible coating effects on storage life and quality of tomatoes. *Journal of Food Science, 59*(3), 568–570. https://doi.org/10.1111/j.1365-2621.1994.tb05563.x.
Quintavalla, S., & Vicini, L. (2002). Antimicrobial food packaging in meat industry. *Meat Science, 62*(3), 373–380. https://doi.org/10.1016/S0309-1740(02)00121-3.
Sapers, G. M., & Sites, J. E. (2003). Efficacy of 1% hydrogen peroxide wash in decontaminating apples and cantaloupe melons. *Journal of Food Science, 68*(5), 1793–1797. https://doi.org/10.1111/j.1365-2621.2003.tb12331.x.
Scramin, J. A., de Britto, D., Forato, L. A., Bernardes-Filho, R., Colnago, L. A., & Assis, O. B. (2011). Characterisation of zein–oleic acid films and applications in fruit coating. *International Journal of Food Science & Technology, 46*(10), 2145–2152. https://doi.org/10.1111/j.1365-2621.2011.02729.x.
Selling, G. W., Woods, K. K., Sessa, D., & Biswas, A. (2008). Electrospun zein fibers using glutaraldehyde as the crosslinking reagent: Effect of time and temperature. *Macromolecular Chemistry and Physics, 209*(10), 1003–1011. https://doi.org/10.1002/macp.200700606.
Sessa, D. J., Mohamed, A., & Byars, J. A. (2008). Chemistry and physical properties of melt-processed and solution-cross-linked corn zein. *Journal of Agricultural and Food Chemistry, 56*(16), 7067–7075. https://doi.org/10.1021/jf800712k.
Shukla, R., & Cheryan, M. (2001). Zein: The industrial protein from corn. *Industrial Crops and Products, 13*(3), 171–192. https://doi.org/10.1016/S0926-6690(00)00064-9.
Siro, I., Devlieghere, F., Jacxsens, L., Uyttendaele, M., & Debevere, J. (2006). The microbial safety of strawberry and raspberry fruits packaged in high-oxygen and equilibrium-modified atmospheres compared to air storage. *International Journal of Food Science & Technology, 41*(1), 93–103. https://doi.org/10.1111/j.1365-2621.2005.01046.x.
Ukuku, D. O., Bari, M. L., Kawamoto, S., & Isshiki, K. (2005). Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of bacterial pathogens from whole melon surfaces to fresh-cut pieces. *International Journal of Food Microbiology, 104*(2), 225–233. https://doi.org/10.1016/j.ijfoodmicro.2005.01.016.
Ukuku, D. O., Geveke, D. J., Chau, L., Bigley, A., & Niemira, B. A. (2017). Appearance and overall acceptability of fresh-cut cantaloupe pieces from whole melon treated with wet steam process. *LWT-Food Science and Technology, 82*, 235–242. https://doi.org/10.1016/j.lwt.-2017.04.033.
Ünalan, İ. U., Korel, F., & Yemenicioğlu, A. (2011). Active packaging of ground beef patties by edible zein films incorporated with partially purified lysozyme and Na2EDTA. *International Journal of Food Science & Technology, 46*(6), 1289–1295. https://doi.org/10.1111/j.1365-2621.2011.02625.x.

Ünalan, İ. U., Arcan, İ., Korel, F., & Yemenicioğlu, A. (2013). Application of active zein-based films with controlled release properties to control Listeria monocytogenes growth and lipid oxidation in fresh Kashar cheese. *Innovative Food Science & Emerging Technologies, 20*, 208–214. https://doi.org/10.1016/j.ifset.2013.08.004.

Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., et al. (2001). Listeria pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews, 14*(3), 584–640. https://doi.org/10.1128/-CMR.14.3.584-640.2001.

Wang, H. J., Gong, S. J., Lin, Z. X., Fu, J. X., Xue, S. T., Huang, J. C., et al. (2007). In vivo biocompatibility and mechanical properties of porous zein scaffolds. *Biomaterials, 28*(27), 3952–3964. https://doi.org/10.1016/j.biomaterials.2007.05.017.

Woods, K. K., Selling, G. W., & Cooke, P. H. (2009). Compatible blends of zein and polyvinylpyrrolidone. *Journal of Polymers and the Environment, 17*(2), 115–122. https://doi.org/10.1007/s10924-009-0128-9.

Yemenicioğlu, A. (2016). Zein and its composites and blends with natural active compounds: Development of antimicrobial films for food packaging. In J. Barros-Velázquez (Ed.). *Antimicrobial food packaging* (pp. 503–513). Academic Press. https://doi.org/10.1016/B978-0-12-800723-5.00041-3.

Zapata, P. J., Guillén, F., Martínez-Romero, D., Castillo, S., Valero, D., & Serrano, M. (2008). Use of alginate or zein as edible coatings to delay postharvest ripening process and to maintain tomato (Solanum lycopersicon Mill) quality. *Journal of the Science of Food and Agriculture, 88*(7), 1287–1293. https://doi.org/10.1002/jsfa.3220.