1 # Development of pectin-eugenol emulsion coatings for
2 inhibition of listeria on webbed-rind melons: a comparative
3 study with fig and citrus pectins

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24 **Abstract**

25 The objective of this study was to employ pectin based antimicrobial coatings for inhibition of
26 *Listeria* on surfaces of whole webbed-rind melons that cause frequent outbreaks of Listeriosis.
27 For this purpose, emulsion-based coatings were developed using citrus pectin (CPEC) or pectin
28 extracted from processing wastes of sun-dried figs (FPEC) and eugenol (EUG). The emulsions
29 of FPEC and CPEC with EUG (droplet size range: 1.99-11.22 μm) were highly stable for
30 minimum 10 days at 10° C. The FPEC-EUG films showed higher flexibility and degree of
31 wettability than CPEC-EUG films. In contrast, CPEC-EUG films had a higher gas barrier
32 performance against oxygen at 50% relative humidity than FPEC-EUG films. The zone
33 inhibition tests showed that FPEC-EUG films are more effective against *L. innocua* than
34 CPEC-EUG films. However, FPEC and CPEC coatings with 2% EUG caused 2.2 and 2.7-
35 decimal inactivation of *Listeria* on Galia melons within 1-weeks, respectively. The pectin
36 coatings with EUG could reduce the risk of Listeriosis from webbed-rind melons.

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38 **Keywords:** Fig pectin, sun-dried figs, processing wastes, eugenol, antimicrobial coating,
39 melon, Listeria

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46 **1. Introduction**

47 The microbial outbreaks originating from fruits that grow on the ground are observed
48 very frequently, as they are in direct contact with potential microbial contaminants such as
49 irrigation water, sewage, manure or fertilizer, and animals (Sapers and Sites, 2003; Chen *et al.*,
50 2012; Ma *et al.*, 2016; De Corato, 2019). The melons are among the most important risk fruits
51 since their stem scar and rough peel provide a unique protective environment for pathogenic
52 bacteria such as *Listeria monocytogenes, Salmonella enterica* and *Escherichia. coli* O157:H7
53 (Sapers and Sites, 2003; Chen *et al.*, 2012) that could easily contaminate the inner edible parts
54 of fruit during processes such as cutting and slicing (Ma *et al.*, 2016). The cantaloupe
55 (muskmelon**,** mushmelon, rockmelon or sweet melon) is a particularly risky melon cultivar
56 since it has a complex webbed rind surface that provides a protective growth medium for the
57 *Listeria spp.* (Behrsing *et al.*, 2003). An outbreak of listeriosis in the United States of America
58 linked to cantaloupe melons in 2011 clearly proved the great risk of webbed rind melons since
59 it caused 33 deaths, 1 miscarriage and 143 hospitalizations (CDC, 2012). In 2018, consumption
60 of *L. monocytogenes*-contaminated cantaloupes caused 7 deaths, 1 miscarriage with a total of
61 22 confirmed cases in Australia (NSW DPI, 2018).

62 Active edible coatings incorporated with antimicrobials have been increasingly
63 employed to inhibit microbial pathogens and to increase the quality of fresh-cut fruits (Rojas-
64 Graü *et al.*, 2009). Edible films of chitosan, alginate and zein incorporated with different
65 natural and chemical agents (e.g., cinnamon oil, allyl isothiocyanate, eugenol, nisin, lauric
66 arginate, ethylenediaminetetraacetic acid) have been applied for coating of whole melons
67 (Chen *et al.*, 2012; Ma *et al.*, 2016; Boyacı *et al.*, 2019). However, studies related to the
68 application of emulsion-based pectin coatings incorporated with natural antimicrobials for
69 coating of whole melons are scarce.

70 The objective of the current work is to employ antimicrobial emulsion-based coatings of
71 pectin from different sources with EUG to eliminate Listeria contaminated on Galia melons
72 (hybrids of cantaloupe and honeydew melons), which have a complex webbed rind surface (as
73 cantaloupe) and a sweet, creamy textured, light yellow to green flesh (as honeydew). Due to
74 its inherent bio-adhesive properties (Farris *et al.*, 2011), pectin might be a suitable coating
75 material to deliver antimicrobials onto fruit surface contaminated with pathogens. The main
76 original feature of this study is that, for the first time, it proposed the development of edible
77 coatings using pectin extracted from wastes arising from the industry of sun-dried fig
78 processing. The fig fruit has long been known for its high soluble dietary fiber content that is
79 formed mainly by pectin (Trad *et al.*, 2014). Turkey, with 78.2 metric tons of production in the
80 2017/18 season, is the largest producer and exporter of sun-dried figs in the world
81 (Anonymous, 2018). The quality of fruits obtained by the classical sun-drying process that
82 starts on trees and continues in fields until reaching the targeted moisture content (18-22%
83 moisture) is highly variable and depends strongly on climate, environmental factors, and
84 agricultural practices. Therefore, a considerable amount of the production is represented by
85 low quality and defected sun-dried figs that, for this reason, can be processed to produce
86 marmalade, molasses, animal feed, or ethanol, depending on their quality and severity of their
87 defects. This work opens new perspectives to fruit industry by employing sun-dried fig
88 processing wastes in production of a value-added product such as pectin, and by
89 characterization and application of obtained pectin films for coating of webbed-rind melons
90 that cause challenging safety problems. The coating procedure developed in this work could
91 be applied to melons at the post-harvest period in packaging houses following classical washing
92 procedures. This kind of an antimicrobial coating procedure may be an additional measure
93 against remaining pathogens at the fruit surface.

94 **2. Materials and methods**

95 ***2.1. Materials***

96 Citrus pectin (P9135, galacturonic acid ≥ 79%, methoxy content ≥ 8%) and eugenol
97 (E51791) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were
98 reagent grade. Purees of sun-dried fig processing wastes (a mixture of highly defected fruits,
99 fruit residues from processing, fruits routinely separated for quality control), all passed from
100 UV inspection for luminescence that indicates aflatoxins, were kindly supplied by KFC Gida
101 A.S. (Izmir, Turkey). The purees were mixed, divided into small portions, and kept at -20° C
102 until used for pectin extraction. The *L. innocua* NRRL-B 33314 (ATCC 1915) was from the
103 culture collection of the microbiology laboratory of the Department of Food Engineering at
104 Izmir Institute of Technology. The Galia melons (*Cucumis melo* var. *reticulatus*) were
105 purchased from a local market in Izmir, Turkey.

106 ***2.2. Methods***

107 **2.2.1. Extraction of fig pectin from waste material**

108 Pectin was extracted by the hot acidic extraction method (Yuliarti et al., 2015) with
109 slight modifications. For this purpose, 50 g of sun-dried fig puree was mixed with 150 mL of
110 a solution of citric acid (CA) at different concentrations (1.0%, 3.0% or 6.0% (w/v)). The
111 mixture was then homogenized for one minute at high speed using a Waring laboratory blender
112 (31BL91, Torrington, CT, USA) equipped with a stainless still jar. The homogenate was first
113 heated to 95° C using a hotplate under continuous stirring (for 1 or 3 hours) and then cooled to
114 room temperature in an ice bath, followed by centrifugation at 5000 × g at 4° C for 20 minutes.
115 The supernatant was collected (extract-1) and the precipitate obtained was extracted again
116 (sample to acid ratio 1:1, CA concentration 1.0%, 3.0% or 6.0% (w/v), extraction at 95° C for
117 30 minutes) to collect the residual pectin. The mixture obtained from the second extraction was
118 centrifuged (extract-2) and it was combined with the extract-1. For the precipitation of pectin

119 in the total extract, 96% ethanol (extract to ethanol ratio 1:2) was mixed with the extract at
120 room temperature, and the mixture was stirred for 30 minutes. The extract-ethanol mixture was
121 then left at 4° C for 24 hours to precipitate the pectin. The precipitated pectin was collected by
122 centrifugation at 9600 × g for 10 minutes. The collected precipitate was then washed twice
123 with ethanol to remove impurities and water as well as to reduce brown pigments. The obtained
124 purified pectin was then dried for 12-18 hours in an oven at 40° C to remove residual ethanol,
125 and then it was brought to ≤ 5% moisture content by lyophilization (Labconco, FreeZone, 6 L,
126 Kansas City, MO, USA).

127 **2.2.2. Molecular properties and composition of extracted pectin**

128 The degree of esterification of the fig pectin was determined by the titrimetric method
129 described by Nazaruddin et al., (2013). The results were expressed as % from the average of
130 three measurements.

131 The galacturonic acid (GA) content was determined by the classical meta-
132 hydroxydiphenyl method spectrophotometrically at 520 nm (Cemeroğlu, 2010). The standard
133 curve was obtained using GA. The average of three measurements was used to calculate the
134 results as % (g GA/100 g of pectin).

135 The soluble protein content of extracted pectin was determined by the Bradford method
136 (1976) using BSA as a standard. The average of triplicate measurements was expressed as g
137 protein/100 g of pectin.

138 The phenolic content of extracted pectin was determined spectrophotometrically at 760
139 nm using the Folin-Ciocalteau's reagent as a reactive compound and gallic acid (GAE) as a
140 standard (Singleton and Rossi, 1965). The average of triplicate measurements was expressed
141 as mg GAE/100 g of pectin.

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143 **2.2.3. Preparation of pectin-EUG emulsion-based films and coatings**

144 For this purpose, 3 g of FPEC or CPEC was suspended in 100 mL of distilled water.
145 The suspension was then heated on a hotplate under continuous stirring at 60° C for 30 minutes.
146 After cooling to room temperature, the solution was homogenized at 10,000 rpm for 1 minute
147 using a homogenizer (Heidolph, Germany, rotor ϕ = 6.6 mm tip). The pH of the mixture was
148 adjusted to 8.0 using 4M NaOH, and it was stirred for 30 minutes to cause de-esterification of
149 pectin. This process intended increasing pectins' negatively charged exposed $–COO^-$ groups
150 that are important to create repulsive forces among oil droplets and increase emulsion stability
151 (Ngouémazong et al., 2015). The pH of the mixture was then slowly lowered to a final value
152 between 3 and 4 with 1M HCl. After that, 0.9 g of glycerol (30% of pectin) was added as a
153 plasticizer of emulsion films, and the mixture was stirred for 15 minutes. Finally, to prepare
154 FPEC-EUG and CPEC-EUG emulsions, EUG was added into the mixture at different
155 concentrations (0.25%, 0.5%, 1%, 2% or 3% (w/w)), and the mixtures were then homogenized
156 at 10,000 rpm for 4 min. The freshly prepared emulsions with desired amounts of EUG were
157 used directly in melon coating studies as described in section 2.2.6 while films used in
158 characterization studies and zone inhibition tests on the agar surface were obtained by the
159 casting method. To obtain pre-cast films, 20 g of the emulsion was cast onto a glass Petri dish
160 (inner diameter 10 cm), which was dried in a controlled test cabinet at 25° C and 50% RH for
161 24 hours.

162 **2.2.4. Stability of pectin-EUG emulsions**

163 The stability of FPEC-EUG and CPEC-EUG emulsions was determined by monitoring
164 the turbidity evolution for 10 days at 10°C. The turbidity values were expressed in nefalometric
165 turbidity units (NTU) using a HACH turbidity meter (2100 AN, USA) and in absorbance units
166 by measuring the absorbance of emulsions at 600 nm. Tests were conducted using two
167 replicates. Zeta potential and particle size of the emulsions were also determined using a
168 NanoPlus DLS Particulate Systems (Micromeritics Instrument Corporation, GA, US).

169 **2.2.5. Antimicrobial activity of films on inoculated agar surface**

170 The antimicrobial activity of freshly prepared pre-cast FPEC-EUG and CPEC-EUG
171 films containing EUG between 0.25% and 2% (w/w) were tested under aseptic conditions by
172 the agar diffusion method as reported by Boyaci et al. (2019) using *L. innocua* as test
173 microorganism. The discs (diameter: 1.3 cm) of pre-cast and dried films were formed at aseptic
174 conditions by a cork borer. Antimicrobial activity test was conducted as two replicates. Nine
175 discs (1 disc was placed per Petri dish) from each film were tested at each replicate. The
176 diameters of the clear zones formed around the discs were measured by a micrometer
177 (Chronos®, UK) and the average zone areas were expressed in cm^2.

178 **2.2.6. Antimicrobial activity of coatings on inoculated webbed-rind melons**

179 The antimicrobial activity of FPEC-EUG and CPEC-EUG coatings with 2% EUG was
180 also tested on inoculated melons. Melons were first washed extensively in tap water, followed
181 by ethanol (70%, w/w) and sterile distilled water. The cleaned melons were then left to dry
182 under the laminar flow hood overnight at room temperature. For the preparation of the
183 inoculum, overnight cultures of *L. innocua* grown in nutrient broth under aerobic conditions at
184 37° C were prepared. One mL of this active culture was transferred to 9 mL of nutrient broth
185 in a tube and incubated at 10° C for 24h to promote the adaptation of the culture to the cold-
186 storage conditions. Two separate zones (4 cm × 4 cm) on each melon's surface were then
187 inoculated by spreading 150 µL of the *L. innocua* culture (10^8 CFU/mL). The inoculated
188 melons were kept under aseptic conditions for 20 min to promote the absorption and drying of
189 the inoculum on the melon surface. Freshly prepared solutions of FPEC-EUG or CPEC-EUG
190 containing EUG at 2% (w/w) and control FPEC or CPEC pectin film solutions (150 µL) were
191 then pipetted onto the inoculated areas (4 cm × 4 cm) of melons and spread homogeneously
192 using a sterile plastic rod. Inoculated melons without film treatment were used as control.
193 Melons were kept for 30 min under laminar flow hood to dry pectin film solutions on their

194 surface (0th day). After that, melons were stored at 10° C and 50% RH for 7 days and
195 enumerated for their *Listeria innocua* counts.

196 Microbiological tests were carried out on the 0th and 7th days. A ten g portion of treated
197 areas (4 cm × 4 cm area framed previously with a marker) of melon rind were excised using a
198 sterile knife. The cut rind pieces were placed into stomacher bags (Thermo Fisher Scientific,
199 Inc., Waltham, MA) containing 90 mL sterile 0.1% (w/w) peptone water and homogenized for
200 150 s using a stomacher (BagMixer ® 400, Interscience, France). The homogenates were then
201 serially diluted with 0.1% w/v peptone water and surface plated on Oxford Listeria Selective
202 Agar (Merck, Darmstad, Germany) with Oxford Listeria Selective Supplement (Merck,
203 Darmstad, Germany). Counting of colonies was carried out after 48-h incubation at 37° C. The
204 counts were performed in triplicate plates for 2 inoculated areas (4 cm × 4 cm) for each
205 treatment (uncoated, FPEC or CPEC coated, and FPEC-EUG or CPEC-EUG coated samples).
206 Microbiological counts were expressed as logarithm (Log) of colony-forming unit per gram
207 (Log CFU g^{-1}) for each treatment.

208 **2.2.7. Tensile properties of films**

209 Tensile strength at break, elongation at break, and Young's modulus were determined
210 using a Texture Analyzer TA-XT2 (Stable Microsystems, Godalming, UK) according to
211 ASTM Standard Method D 882-02 (ASTM, 2002). The dried films were cut into 5 mm wide
212 and 80 mm long strips. The initial grip distance was 50 mm, and the crosshead speed was 50
213 mm/min. At least eight replicates of each film were tested. The thickness of the films was
214 measured by using a micrometer (Chronos®, UK).

215 **2.2.8. Scanning Electron Microscopy (SEM) of films**

216 The cross-sectional morphologies of pectin film samples with and without eugenol were
217 examined by using SEM (250 Quanta FEG, FEI Company, United States). The films were

218 prepared by crushing after freezing in liquid nitrogen. Specimens were gold-coated with a
219 sputter coater (Emitech K550X, Quorum Technologies Inc.UK) under 15 mA for 60 s.

220 **2.2.9. Surface wettability**

221 Water contact angle measurements were performed on both the pristine (control) pectin
222 films (FPEC and CPEC) and the EUG-containing pectin films (FPEC-EUG and CPEC-EUG)
223 using an optical contact angle apparatus (OCA 15 Plus, Data Physics Instruments GmbH,
224 Filderstadt, Germany) equipped with a high-resolution CCD camera and a high-performance
225 digitizing adapter. SCA20 software (Data Physics Instruments GmbH, Filderstadt, Germany)
226 was used for the image capturing and contact angle determination. Rectangular specimens (3
227 $\times 1.5$ cm^2) were kept flat throughout the analysis, and the contact angle of water in air (θ, deg)
228 was measured by gently dropping a droplet of 4.0 ± 0.5 mL of Milli-Q water (18.3 MV cm)
229 onto the substrate at $23 \pm 1°$ C and $50 \pm 2\%$ relative humidity (RH).

230 **2.2.10. Oxygen Transmission Rate (OTR)**

231 The oxygen barrier properties of the films were assessed using a Multiperm permeability
232 analyzer (Extrasolution Srl, Capannori, Italy) equipped with an electrochemical sensor. Pectin
233 films were sandwiched between two aluminum-tape masks, allowing a surface of 2.5 cm^2 to
234 be exposed to the permeation of oxygen. The oxygen transmission rate (OTR, cm^3 m^{-2} 24h^{-1})
235 was determined according to the standard method of ASTM F1927, with a carrier flow (N_2) of
236 10 mL min^{-1} at 23° C and 50% relative humidity (RH), and at 1 atm pressure difference on the
237 two sides of the specimen. Each OTR value was from three replicates.

238 **2.2.11. Statistical analysis**

239 Results were analyzed for significance by using variance analysis (one way-ANOVA)
240 and Fisher post-test using Minitab (ver.16.2.0.0, Minitab Inc., United Kingdom). The
241 differences were considered significant if $P \leq 0.05$.

242 **3. Results and discussions**

243 **3.1. Extraction yield and some characteristics of fig pectin from waste material**

244 The extraction yields and some molecular properties of pectin obtained from sun-dried
245 fig processing wastes at different extraction conditions are given in Table 1. The yield of pectin
246 ranged between 6.70% and 9.12% was increased by the increase of CA concentration. In
247 contrast, the increase of extraction time from 1h to 3h at a given CA concentration did not
248 cause a significant increase in the pectin yield (P >0.05). Thus, the application of 3h extraction
249 is not beneficial to improve extraction yield. The basic molecular properties, GA and DE, of
250 fig pectin ranged between 18.6-24.6% and 57.4-79.3% depending on the severity of extraction,
251 respectively. The most effective factor on the molecular properties was CA that caused a
252 concentration-dependent reduction in GA and DE of pectin. The increase of the extraction
253 period from 1h to 3h at a certain concentration of CA had no significant effect on GA of pectin
254 (P >0.05). The increase of the extraction period did not also affect DE in presence of 1 and 3%
255 CA while this caused a significant reduction in DE in the presence of CA at 6% (P <0.05). The
256 overall results suggested that extraction at 95° C with 3 or 6 % CA for 1h could be employed
257 to obtain fig pectin yield between 8.0 and 9.0 %. In the current work, to minimize modifications
258 in pectin chain length, mild extraction at 3% CA for 1h was applied rather than extraction at
259 6% CA for 1h.

260 In the literature, studies related to pectin extraction from sun-dried fig processing
261 wastes are scarce. However, the extraction yield obtained at 95° C with 3% or 6% CA for 1h
262 from sun-dried fig waste in the current work was higher than the pectin yield of 6% obtained
263 by Garibzahedi et al. (2019), who extracted peels of fresh figs with CA-acidified hot water at
264 90° C for 1h. Moreover, the extraction yield of Garibzahedi et al. (2019) from ultrasound-
265 microwave assisted extraction (11.7%) was higher than those obtained in the current work with

hot acidic extraction. These results clearly showed that the processing wastes of fresh or dried figs could be evaluated as a source of pectin.

3.2. Stability of pectin-EUG emulsions

The 3% solutions of FPEC or CPEC formed highly turbid and stable emulsions with EUG at concentrations between 0.25 and 2%. This was demonstrated by the stable spectrophotometric absorbance measurements at 600 nm for both FPEC-EUG and CPEC-EUG emulsions, that cold-stored at 10° C for 10 days (see Supporting Information, Table S1). The turbidity of all FPEC-EUG and most of CPEC-EUG emulsions were also not measurable (> 10000 NTU). The only measurable NTUs were those obtained from CPEC at 0.25% EUG and 1% EUG, with values that remained stable during 10 days of cold storage in the ranges 4000-5000 NTU and 8000-10000 NTU, respectively (see Supporting Information, Table S1). The stability of FPEC-EUG and CPEC-EUG emulsions were also proved by their limited changes in droplet size and zeta potential values during 10 days of cold storage (Table 2). The initial droplet size of FPEC-EUG emulsions was significantly lower than that of CPEC-EUG emulsions. This result seems to indicate a better emulsifying capability of FPEC than CPEC, which could be ascribed to the high protein content of FPEC coming from the seeds of fig (FPEC and CPEC contain 15.0 and 6.2 g protein/100 g pectin, respectively). No significant differences were detected in the droplet size (3.46 to 10.08 μm) for both types of emulsions between 3 and 10 days of cold storage, which suggests that FPEC and CPEC behaved in a similar way in terms of emulsion stability. It is also important to note that both emulsions remained stable and they did not show any visually detectable phase separation minimum 6 weeks at 10°C. Moreover, FPEC-EUG emulsions showed slightly to moderately lower zeta potential values than CPEC-EUG emulsions, possibly due to a lower GA content (means less $-COO^{-}$), but higher protein content (might mask negative charges) of FPEC than CPEC. Further long term storage stability tests are needed to understand the feasibility of

291 commercializing ready-to-use emulsion preparations. However, it should be noted that the best
292 performance (homogeneity and effectiveness) from antimicrobial emulsion-based coatings
293 with volatile essential oils are obtained when their emulsions are prepared freshly before each
294 application.

295 **3.3. Antilisterial activity of films in zone inhibition test**

296 The results of the zone inhibition tests with films containing EUG between 0.25% and
297 2% against *L. innocua* are displayed in Figure 1. The minimum amounts of EUG in films that
298 yielded clear zones around the discs of FPEC and CPEC films were 0.25% and 0.5%,
299 respectively. However, the clear zones at the indicated EUG concentrations were observed only
300 in 8 out of 18, and 14 out of 18 discs tested for FPEC and CPEC films, respectively, due to the
301 fact that the inhibitory concentration was not reached in a homogeneous manner all around the
302 discs. However, the 'no zone discs' were eliminated when EUG concentrations were increased.
303 The FPEC-EUG films formed significantly greater zone areas (5 and 1.6 fold) than CPEC-
304 EUG films at 0.5% and 1% EUG concentrations, respectively. Thus, it is clear that FPEC-EUG
305 emulsion-based films performed better than CPEC-EUG films in terms of EUG release at the
306 test conditions used in the experiment. However, both CPEC-EUG and FPEC-EUG films
307 showed similar antimicrobial activities when the concentration of the essential oil was
308 increased to 2%, a critical concentration that provided an excessive amount of EUG in both
309 films for effective inactivation of Listeria. Thus, the optimal EUG concentration of 2% was
310 used in films tested on inoculated melons.

311 **3.4. Antilisterial activity of films at inoculated webbed melon surfaces**

312 The results of *L. innocua* counts conducted at the beginning and at the end of 1-week
313 cold storage (at 10° C) for uncoated, and FPEC, CPEC, FPEC-EUG, and CPEC-EUG coated
314 inoculated webbed melons are presented in Figure 2. The Listerial counts of uncoated control

315 melons and control CPEC coated melons were not significantly different at 0th day (P >0.05)
316 while CPEC coated melons showed significantly lower Listerial counts at 7th day of cold
317 storage ($P < 0.05$). In contrast, it is important to note that melons coated with control FPEC
318 films gave significantly lower Listerial counts than uncoated controls at both 0th and 7th days
319 of cold storage ($P < 0.05$). This finding suggested that the pectin extracted from sun-dried fig
320 wastes showed an inherent antimicrobial activity that might originate from the antimicrobial
321 activity of polyphenols associated with this hydrocolloid (Amessis-Ouchemoukh *et al.*, 2017).
322 Polysaccharides like pectin interact with polyphenols via their polar groups (e.g. acetal,
323 hydroxyl or carboxyl) and bind phenolic –OH groups with H-bonds and van der Waals forces
324 (Palafox-Carlos *et al.*, 2011). Thus, polyphenols could not be separated effectively from pectin
325 by classical ethanol precipitation and washing cycles applied during pectin purification.
326 Gharibzahedi et al. (2019) reported that pectin from peels of fresh common figs contained 3 g
327 GAE equivalents of total phenols per 100 g of pectin (d.w.). In the current study, the total
328 phenolic content of extracted FPEC was almost 2 fold higher than that of CPEC (as 1.03 and
329 0.55 g GAE/100 g the of pectin (d.w.), respectively). Thus, these results supported that the
330 inherent antimicrobial activity of FPEC control film could be due to its high polyphenol
331 content. Meanwhile, the antimicrobial activity on coated melons against *L. innocua* increased
332 significantly by the application of pectin-EUG coatings. It is important to note that on the 0th
333 day, the Listerial counts of melon surfaces coated with CPEC-EUG and FPEC-EUG films were
334 1.5 and 1.3 decimals (D) lower than the Listerial counts obtained from uncoated melon
335 surfaces, respectively. Moreover, the decimal differences between Listeria counts of uncoated
336 and CPEC-EUG or FPEC-EUG film-coated samples increased to 2.7 and 2.2 D by 1-week cold
337 storage, respectively. Although the Listerial counts of melons coated with CPEC-EUG were
338 significantly lower than those coated with FPEC-EUG at the end of 7th days ($P \leq 0.05$), the
339 antimicrobial performance on webbed-rind melon surfaces of the pectin coatings from different

340 origin was comparable. After 1-week of storage, the sharp smell of EUG in melons with CPEC-
341 EUG and FPEC-EUG coatings reduced considerably due to the evaporation of essential oil
342 from the pectin films. Thus, it seemed that the increased decimal reduction in Listeria by
343 storage was related mainly to the death of bacteria damaged at the initial stages of the coating
344 when pectin films had contained high concentrations of EUG. Further studies are needed to
345 determine the kinetics of EUG loss from both types of pectin films at different temperatures,
346 and to understand the duration of antimicrobial effectiveness following application of films.

347 In the literature, studies related to the use of pectin coatings with essential oils on whole
348 webbed rind melons are scarce. However, Boyaci *et al* (2019) reported that zein films with 2%
349 EUG caused almost 3.3 D reduction in listerial counts of some melon cultivars (Santa Claus
350 and Crenshaw) having smooth to slightly rough rind surface within 1-week of cold storage.
351 This result indicated the possibility that the pectin emulsion coatings with EUG are slightly
352 less effective on whole melon surfaces than zein coatings with EUG. However, both films
353 should be compared for the same film thickness on webbed rind melons that provide a better
354 hiding and growth medium for Listeria than smooth rind melon cultivars (Behrsing *et al.*,
355 2003). Ma *et al.* (2016) also obtained > 3 log CFU/cm^2 reduction of *Escherichia coli* O157:H7
356 and *Listeria monocytogenes* on whole cantaloupes immediately after applying chitosan coating
357 with 0.1 % lauric arginate, 0.1 % ethylenediaminetetraacetic acid and 1% cinnamon oil on their
358 surface. Moreover, Zhang et al. (2015) also achieved effective inhibition of different pathogens
359 including *Listeria monocytogenes* on cantaloupes using alginate films with 2% cinnamon bark
360 oil. All these studies clearly showed the good potential of using antimicrobial coatings to
361 increase the safety of whole webbed rind melons. However, further studies are needed to
362 determine the long-term effects of edible coatings on the quality attributes (e.g., surface color,
363 texture and sensory properties) of melons.

364 **3.5. Mechanical properties of films**

365 Mechanical properties of films obtained from CPEC-EUG and FPEC-EUG emulsions
366 at different EUG concentrations are shown in Table 3. At all conditions, CPEC and CPEC-
367 EUG films showed significantly higher tensile strengths (17.9 to 51-fold) and Young's
368 modulus than FPEC and FPEC-EUG films. However, it is important to note that the FPEC
369 films were much more flexible, and they showed 2.4 to 4.3-fold higher elongation at break than
370 CPEC films. It should also be reported that the increase of EUG concentration between 1% and
371 3% did not cause a considerable change in the mechanical properties of both types of films.
372 These results clearly showed the different film making characteristics of CPEC and FPEC.

373 **3.6. Morphology of pectin films**

374 SEM micrographs of cross-sectional morphology for FPEC and CPEC films with and
375 without EUG are shown in Figure 3. Control films prepared with FPEC and CPEC both had a
376 dense appearance (Figures 3A and 3B). However, control CPEC films exhibited a more
377 uniform morphology than control FPEC films. FPEC films, in particular, contained some
378 aggregates, possibly arising from insoluble proteins originated from seeds of fig fruit. In
379 contrast, FPEC-EUG and CPEC-EUG films revealed a morphology characterized by an
380 extensive distribution of droplets and void pores (Figure 3C and D). It appeared that part of the
381 EUG remained in the films as emulsion droplets, while some other EUG droplets evaporated
382 to form some voids (pores). Noteworthy, the EUG emulsion droplets were distributed
383 homogenously along the film matrix, with no signs of accumulation close to the film surface.
384 Nisar et al. (2018) observed similar morphologies in citrus pectin films incorporated with clove
385 bud essential oil (CEO). However, these authors observed coalescence of CEO droplets close
386 to the film surface, which was ascribed to phase separation between pectin and the essential
387 oil, as CEO concentration was increased from 0.5% to 1% or 1.5% (Nisar *et al.*, 2018).

388 **3.7. Surface wettability of films**

389 The results of the contact angle analysis are summarized in Table 4. The films of pectin
390 exhibited a completely different behavior depending on the origin. Pristine FPEC films showed
391 a typical wetting behavior (θ ~ 27°), that is, water molecules rapidly interacted with the film
392 surface due to high chemical affinity. The marked polar character owing to both the properties
393 of the galacturonic skeleton (side chain frequency, sugar profile and chain length) and the
394 protein fraction drove water molecules to spread on the surface as soon as the droplet touched
395 the surface, insomuch as, after a few seconds, θ ~ 10° (see Supporting information, movie 1).
396 As expected, the addition of EUG yielded a significant increase in θ (~ 54°) due to the
397 hydrophobic character of EUG that made the FPEC film surface more apolar. In turn, this
398 reduced the intensity of the spreading phenomenon of water molecules on the film surface (see
399 Supporting Information, movie 2).

400 A completely different scenario was observed for the films of pectin derived from
401 citrus. Oppositely to the pectin films from fig, CPEC films had an unexpectedly high water
402 contact angle (θ ~ 83°). In addition, absorption rather than spreading of water molecules
403 occurred on the surface (see Supporting information, Figure 1S and movie 3). A plausible
404 explanation for this behavior can be found in the composition of this type of pectin. Pectin from
405 citrus had a high GA content (≥ 79%). It appeared that this played a role in the structural
406 organization of pectin molecules, with most likely an extensive hydrogen bonding at
407 intermolecular level, which yielded a dense, less permeable network. For this reason, water
408 molecules were less prone to wet the film surface compared to the surface of fig pectin films.
409 In the case of fig pectin, the lower GA content (20.16%), but higher protein content than citrus
410 pectin, probably did not allow the formation of a dense and stable organization at a molecular
411 level. The addition of EUG (CPEC-EUG) led to a significant decrease of θ (~ 73°) (see
412 Supporting information, movie 4). In line with the above considerations, it is plausible that the
413 antimicrobial agent somehow perturbed the original CPEC network organization working as a

414 pseudo-plasticizer, thereby disrupting (at least partly) the inherent hydrogen-bonding pattern
415 in the pristine samples.

416 **3.8. Oxygen permeability characteristics of films**

417 The different chemical composition and physical properties of films obtained from
418 citrus and fig pectins were also reflected in the barrier properties against oxygen. As shown in
419 Table 4, the best barrier performance was observed for the CPEC sample, with an average OTR
420 value slightly below 3 mL / (m^2 24h). This low OTR value can be ascribed to the extensive
421 hydrogen bonds formed by carboxyl and hydroxyl groups of GA units in citrus pectin film. The
422 addition of EUG (CPEC-EUG) impaired the above performance due to the negative effect on
423 the inherent organization as well as because EUG has a hydrophobic character, leading to a
424 final OTR value of ~ 13 mL / (m^2 24h).

425 Films obtained from fig pectin showed a lower barrier performance against oxygen.
426 Pristine FPEC films had an average OTR value of ~ 14 mL / (m^2 24h), very similar to the OTR
427 value of CPEC films with EUG. The lower OTR performance of pristine films of FPEC
428 compared to CPEC can be explained in consideration of the low GA content and high amount
429 of denatured protein fraction of FPEC. All of these parameters contribute to a lower barrier
430 performance due to a less extensive hydrogen bonding among fig pectin molecules at intra-
431 and intermolecular level. After the addition of EUG, FPEC films exhibited an OTR value more
432 than three times higher than the pristine films. Also, in this case, EUG acted alike a plasticizer,
433 that is, it worsened the original hydrogen bonding pattern and increased the free volume of the
434 main biopolymer phase, hence allowing a higher oxygen flux across the film thickness. From
435 a practical point of view, coatings obtained from fig pectin might be more suitable for the
436 intended fruit coating application because the deposition of the coating, while slowing down

437 the respiration rate of the fruit, could still allow oxygen to diffuse into the food, thus avoiding
438 initiation of anaerobic respiration of the product.

439 **4. Conclusions**

440 This study clearly showed the good potential of pectin-EUG emulsion-based edible
441 coatings to reduce the risks associated with contaminated *Listeria* on webbed-rind melons. The
442 antimicrobial emulsions developed using pectin extracted from sun-dried fig processing wastes
443 and commercial citrus pectin showed similar antilisterial effectiveness on coated webbed-rind
444 melons. However, fig pectin films differed from citrus pectin films in terms of critical EUG
445 concentrations to achieve antimicrobial properties, and different physical, mechanical and gas
446 barrier properties that could be exploited to increase the applicability of the developed films on
447 alternative melon cultivars (or crops) that had variable rind characteristics and physiological
448 properties. This work is an example of the valorization of agro-industrial wastes to obtain
449 alternative food hydrocolloids with different functional and technological characteristics.

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457 the Ph.D. student Elif Çavdaroğlu.

458 **Ethical guidelines**

459 Ethics approval was not required for this research.

460 **Data availability statement**

461 Research data are not shared.

462 **Conflict of interest**

463 There are no conflicts of interest in this study.

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531 **Figure legends**

532

533 Figure 1. Zone-inhibition based antimicrobial activity of FPEC-EUG and CPEC-EUG films on
534 *L. innocua*. Data with different letters are significantly different ($p \leq 0.05$).

535

536 Figure 2. Antimicrobial effects of FPEC-EUG and CPEC-EUG coatings with 2% EUG on *L.*
537 *innocua* inoculated onto whole Galia melons during cold storage at 10 ºC. Data with different
538 letters are significantly different ($p \leq 0.05$).

539

540 Figure 3. Effect of EUG on the cross-sectional morphology of pectin films: (A) FPEC; (B)
541 CPEC; (C) FPEC-EUG (2%); (D) CPEC-EUG (2%) (Magnification: 10000 ×).

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