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UV-B radiation on lemons enhances antifungal activity of flavedo extracts against *Penicillium digitatum*

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1 **UV-B radiation on lemons enhances antifungal activity of flavedo extracts**
2 **against *Penicillium digitatum***
3
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23 **ABSTRACT**

24 UV-B radiation (UVBR) treatment, previously standardized in our laboratory, contributes
25 to prevent green mold caused by *Penicillium digitatum* on postharvest lemons. Here,
26 antifungal activity of flavedo extracts from irradiated and non-irradiated lemons was
27 assayed against *P. digitatum*; also, compounds that could be responsible for this action
28 were searched. The flavedo extracts from UV-B exposed lemons (UVBLE) exhibited a
29 higher antimicrobial activity than extracts from control lemons (CLE). Conidia treated with
30 UVBLE showed a time dependent inhibition of germination and oxygen consumption, a
31 markedly increase in ROS and TBARS production and in membrane permeability. By
32 chemical analysis of lemon extracts, two fractions (A and B) that responded to the
33 irradiation were detected, showing a decrease in Fraction A and an increase in Fraction B.
34 Fraction B showed higher antioxidant and antifungal activities in comparison to Fraction A.
35 Both fractions were complex samples enriched in flavonoids. The specific composition of
36 each fraction could explain the difference in the biological activities, and also the higher
37 efficiency of UVBLE to inhibit *in vitro* the pathogen compared to CLE. Based on our
38 results, UV-B radiation treatment can intensify natural defenses of flavedo lemon by
39 eliciting phenolic compounds synthesis, among other processes.

40

41 **KEYWORDS**

42 antimicrobial activity; flavonoids; green mold; lemon; UV-B radiation

43

44 **1. INTRODUCTION**

45 Green mold, caused by *Penicillium digitatum* Sacc., is the most common postharvest
46 disease of citrus fruit worldwide, affecting an average of 5-8% of the total handled fruit
47 (Eckert and Brown, 1986; Eckert and Eaks, 1989). Synthetic fungicides, such as imazalil
48 and thiabendazole, are widely used to control this pathogen (Martínez-Jávega et al., 1995).
49 The continuous use of commercial fungicides has resulted in the appearance of resistant
50 fungal strains (Latorre et al., 1994). Hence, there is an urgent need to control citrus
51 postharvest diseases by alternative technologies to replace or reduce the use of synthetic
52 fungicides. Some natural products isolated from plants exert antifungal activity and could
53 be good candidates for that purpose (Grayer and Kokubun, 2001).
54 Natural resistance in fruit and vegetables can be elicited by physical treatments, such as
55 heat (Ballester et al., 2013, Infla and Booyakiat, 2010, Kim et al., 1991), low temperature
56 (Leyva et al., 1995; Rab et al., 2012), UV-C radiation (D'Hallewinet et al., 2000; Stevens et
57 al., 2005), and mechanical injury (Ballester et al., 2013; Godoy et al., 2001). In spite UV-C
58 has been broadly studied to counteract rots on fruit and vegetables during postharvest and
59 storage (Arcas et al., 2000; Ben-Yehoshua et al., 1992; Del Río et al., 2004; Droby et al.,
60 1993; Haro-Maza and Guerrero-Beltrán, 2013), this radiation can cause significant damage
61 in the citrus skin, such as browning (D'Hallewinet al., 1999). A less harmful alternative is
62 the UV-B radiation (UVBR), although few reports are available concerning to its effect on
63 postharvest fungal decays of citrus fruit (Ruiz et al., 2016; Yamaga et al., 2016).
64 UVBR is a small fraction of the solar spectrum (280-315 nm) which can elicit a wide range
65 of responses in plants such as changes in gene expression, physiology, secondary
66 metabolite accumulation and morphology (Ballaré et al., 2011; Kostina et al., 2001; Robson

67 et al., 2014). UVBR is a positive modulator in plant defense, increasing plant resistance to
68 herbivores and pathogens, due to changes in phenylpropanoid-derivatives, including
69 flavonoids and other soluble phenolic compounds (Ballaré, 2014). The stimulation by
70 UVBR of phenylalanine ammonia lyase (PAL), a branch point enzyme between primary
71 (shikimate pathway) and secondary (phenylpropanoid pathway) metabolisms, has been
72 reported for various plant species (Gleitz et al., 1991; Hadwiger and Schwochau, 1971;
73 Sarma and Sharma, 1999; Treutter, 2006). Among phenolic compounds, flavonoids present
74 antimicrobial activity against *Phytophthora*, *Geotrichum*, *Colletotrichum* and *Penicillium*
75 species as tested *in vitro* (Del Río et al., 1998; Johann, 2003; Johann et al. 2007).
76 Moreover, flavonoids antioxidant capacity was described, being able to diminish tissue
77 levels of reactive oxygen species (ROS) (Brunetti et al. 2013; Treutter, 2006). Likewise,
78 Lagha-Benamrouche and Madania (2013) established a relation between phenolic
79 compounds content and antioxidant activity of orange peel.
80 Flavedo is the outer layer of citrus fruit (Ağçam and Akyıldız, 2014) which concentrates
81 natural defenses, including phenolic compounds, to counteract the fungal infections
82 (Johann et al., 2007; Ortuño et al., 2011). UVBR increases the content of both soluble
83 (flavonoids and UV-B absorbing compounds) and insoluble (lignin) phenolic compounds in
84 the epidermal tissues, constituting natural sunscreens (Hilal et al., 2004; 2008; Prado et al.,
85 2012). Interdonato et al. (2011) showed that the 3 min UVBR dose induces changes in both
86 carbohydrate content and distribution pattern in albedo and flavedo of postharvest lemons.
87 In addition, a UV-B non-germicide 3 min exposure applied on postharvest lemons induces
88 metabolic and anatomical changes in fruit peel, representing an acclimation response that
89 enhances resistance against *P. digitatum* Sacc. (Ruiz et al., 2016). Here, the antifungal

90 activities of irradiated and non-irradiated lemon flavedo extracts were assayed against *P.*
91 *digitatum* and the phenolic compounds that could be responsible for this antifungal action
92 were pursued.
93

94 2. MATERIALS AND METHODS

95 2.1. Plant material

96 Mature lemons (*C. limon* (L.) Burm (cv. Eureka)) of uniform size and appearance, were
97 hand collected from commercial orchard (San Pablo, Tucumán, Argentina), an area
98 corresponding to foothill region. Selected fruit were harvested from the superficial inner
99 portion of the North facing half of the canopy (semi-sun fruit) at around 150 cm above the
100 soil and marked on its sunny face. Lemons were collected during the austral winter season
101 (June to August). After harvesting, fruit were transported to laboratory, washed with
102 distilled water and stored at 25°C for 24 h before UV-B irradiation.
103 In order to characterize phenolic compounds by HPLC, lemons were sampled from twelve
104 geo-referenced trees. From each plant, a pair of adjacent lemons was taken from a same
105 branch, to be used as control and UV-B exposed lemons.

106 2.2. Exposure of postharvest lemons to UV-B

107 24 h after harvest, the lemon sunny faces were exposed to 3 min of UV-B radiation from a
108 distance of 50 cm (22000 Jm^{-2} d^{-1}), using UV-B lamps (Q-Panel 313, Cleveland, USA).
109 Then, lemons were stored at 25ºC and 65% of relative humidity in a dark chamber to
110 minimize any possible photo-reactivation processes (Stevens et al., 2005). Control non-
111 irradiated lemons were stored in the same conditions.

112 2.3. Preparation of flavedo extracts

113 24 h after UV-B exposure, i.e. 48 h postharvest, flavedo tissue slides (~2mm thickness)
114 were cut from lemons. Flavedo of non-exposed lemons were simultaneously sampled. Peel
115 slides were stored at -20°C before extract preparation. Flavedo extracts were obtained using
116 100 mg tissue samples per 1 ml of acidified methanol (MeOH:H_2O:HCl, 79:20:1) and
117 incubating in darkness at 40°C during 12 h (adapted from Hilal et al., 2008). Extracts were
118 dried at 40°C at low pressure conditions and stored at -20°C.
119 For HPLC characterization, flavedo extracts were prepared from single lemons at a
120 concentration of 1g of FW per 4 mL of acidified methanol and filtered by 0.45 μm
121 microclar membrane (Waters, Milford, MA, USA).

122 **2.4. Conidia assays**

123 **2.4.1. Conidial suspension preparation**

124 An isolate of *P. digitatum* was obtained from naturally infected citrus fruit (Tucumán,
125 Argentina) and grown on potato dextrose agar (PDA) dishes, pH 5.5, at 24ºC for 7 d. A
126 protocol adapted by Cerioni et al. (2012) was followed to prepare 10^6 conidia mL^{-1}
127 suspensions.

128 **2.4.2. Determination of mycelial growth in the presence of flavedo extracts**

129 Dried flavedo extracts were resuspended with potato dextrose agar (PDA) obtaining
130 increasing final concentrations (between 0.0375 to 21 mg DW mL^{-1}) to prepare 5 mL
131 plates. On each plate, 5 µl of 10^6 conidia mL^{-1} suspension were spotted. The colony
132 diameters were measured after 5 d of incubation at 24°C. In parallel, conidia were spotted
133 on PDA without extract.

134 **2.4.3. Treatment of conidia with lemon flavedo extracts**

135 Conidial suspensions were treated with 15 mg DW mL^{-1} of flavedo extracts in potato
136 dextrose broth (PD) at 24°C for increasing exposure times (0.5 to 24 h). In parallel, conidia
137 were incubated with sterile distilled water. Treated conidia were evaluated for germination,
138 ROS production, TBARS production, oxygen consumption and membrane permeability, as
139 follows.

140 **2.4.4. Conidia germination**

141 After treatments, conidia were centrifuged at 2000 g for 5 min, washed three times and
142 resuspended with PD to a final concentration of 10^6 mL^{-1} in microwell plates. Germination
143 was monitored for 36 h, using optical microscope Olympus BX51TF (Olympus Co.,
144 Tokyo, Japan). Conidia were considered germinated when germination tubes were at least
145 twice as long as conidia diameters (Xiaoping et al. 2007). Germination percentage was
146 calculated from the obtained values.

147 **2.4.5. Reactive oxygen species (ROS) determination**

148 ROS content was determined on aliquots of treated conidia with the H_2DCFDA probe, as
149 adapted by Cerioni et al. (2010) from other reports (Davidson et al., 1996; Halliwell and
150 Whiteman, 2004). Briefly, treated conidia (5.10^7 conidia mL^{-1}) were washed and exposed to
151 10 μM H_2DCFDA dissolved in dimethylsulfoxide. Then cells were broken by passing them
152 three times through a French press at 89635 kPa. Fluorescence intensity (emission at 525
153 nm; excitation at 488 nm) was measured on aliquots of broken conidia using ISS-PCI
154 spectrofluorometer (Champaign, IL, USA). The fluorescence was normalized by protein
155 concentration.

156 **2.4.6. Thiobarbituric acid-reactive species (TBARS) determination**

157 Aliquots of treated conidia were washed three times and then broken by passing them three
158 times through a French press at 13 000 psi. TBARS were determined as described by Rice-
159 Evans et al. (1991). Briefly, 1 mL of broken conidia was precipitated with 1 mL of 20%
160 TCA (w⁄v) at 4°C and centrifuged at 19800 g for 5 min. The resulting supernatant was
161 mixed with 2 mL of a saturated solution containing 0.65% thiobarbituric acid dissolved in
162 HCl and 10 mM butylated hydroxytoluene dissolved in ethanol. Then, the mix was heated
163 at 100°C for 60 min. The concentration of TBARS was calculated using values of
164 absorbance at 535 nm and a molar extinction coefficient of 156 $mmoL^{-1}cm^{-1}$, and expressed
165 as pmol mg^{-1} protein.

166 **2.4.7. Oxygen consumption measurement**

167 The oxygen consumption of treated conidia was determined after washing (three times) and
168 resuspending with sterile distilled water at a concentration of 5.10^7 conidia mL^{-1}. Oxygen
169 consumption was measured in a thermostatic chamber at 25°C coupled to oxygen detector
170 (Oxygraph-2k, Oroboros, Austria) for 30 min, using 2 mL of the concentrated conidial
171 suspension. Relative oxygen consumption rate was calculated as a percentage of the control
172 treated with sterile distilled water.

173 **2.4.8. Membrane integrity determination**

174 Membrane integrity determination was performed by the uptake of Sytox Green adapted
175 from Muñoz and Marcos (2006). The technique was applied as follows: treated conidia
176 suspension was centrifuged, washed, resuspended and incubated with 0.2 µM Sytox Green
177 for 5 min in darkness. Conidia were visualized under a fluorescence microscope Olympus
178 BX51TF equipped with an Olympus digital camera, QColor5 (Q-imaging, Surrey, BC,
179 Canada). The emission of fluorescence was examined and photographed using a filter set

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180 for excitation of 450-490 nm and for emission of 515-565 nm. In each case, at least 100
181 conidia were counted under visible light and blue light, and the percentage of fluorescent
182 conidia was calculated.

183 **2.4.9. Protein determination**

184 Protein content was determined by the Lowry et al. (1951) method, using bovine serum
185 albumin (BSA) as a standard.

186 **2.5. Chemical characterization of flavedo extracts**

187 **2.5.1. Chromatographic profile by HPLC-DAD**

188 The flavedo extracts from single selected lemons were analyzed by HPLC coupled to a
189 diode array detector (HPLC-DAD), using an analytical X Bridge^{TM} C18 column (5 µm, 4.6
190 x 150 mm) and a chromatographer Waters 1525 (Waters Corporation, USA). The HPLC
191 analysis was performed using a linear gradient solvent system consisting of water:acetic
192 acid 0.1% (A) and methanol:acetic acid 0.1 % (B) as follows: 90% A to 43% A over 45
193 min, followed by 43 to 0% A from 45.0 to 60 min and 100% B from 60 to 65 min. The
194 flow rate was 0.5 mL/min and the volume of injected sample was 20 µL. The
195 chromatograms were visualized with Empower 2^{TM} software (Waters Corporation, USA) at
196 330 nm. The assays were conducted twelve times with two repetitions for each replica.

197 **2.5.2. Fractionation of phenolic compounds**

198 Flavedo extracts were submitted to semipreparative HPLC-DAD to isolate the fractions
199 with stable behavior in response to irradiation. For this, a Luna C18 semi-preparative
200 column (Phenomenex, USA) 5µ (250 x 10 mm) was used. The injection volume was 50 µL.
201 The elution was carried out with a mobile phase formed by A: acetic acid 0.1% and B:
202 methanol: acetic acid 0.1%, using a gradient as follows: 90% A to 43% A over 45 min,

203 followed by 43 to 0% A from 45 to 60 min and 100% B from 60 to 65 min. The
204 chromatograms were visualized with Empower 2^{TM} software (Waters Corporation, USA) at
205 330 nm.

206 **2.5.3. Evaluation of the antioxidant and antifungal activities of HPLC fractions**

207 The evaluation of the antioxidant activity of HPLC fractions was carried out according to
208 Re et al. (1999) using the ABTS$^{\bullet+}$ radical discoloration. The percentage of discoloration
209 was calculated according the following formule:

210 $$\% \ discoloration = (A0 - AS / A0) \times 100$$

211 where A0 is the Abs at 734 nm without extract and AS is the Abs at 734 nm of the sample.
212 The values represent the µg gallic acid equivalents (GAE) mL^{-1} required to discolor 50% of
213 the ABTS radical.

214 In order to evaluate antifungal activity of selected HPLC fractions, different amount of
215 samples (between 0.25 and 50 mg L^{-1}) were dried by SpeedVac (Savant, Thermo Scientific
216 USA) and resuspended in conidia suspension (10^{6} conidia mL^{-1}). The suspensions were
217 incubated at 25°C for 12 h in darkness. Then, 5 µL of each suspension were spotted on
218 Petri dishes with PDA medium. After 5 d of incubation at 25°C, colony diameters were
219 measured.

220 **2.5.4. Identification of compounds by HPLC-DAD-MS/MS**

221 The polyphenols analysis of selected fractions was assessed in the Instituto Superior de
222 Investigación, Desarrollo y Servicios en Alimentos (ISIDSA) of the National University of
223 Córdoba, by HPLC-DAD-MS/MS (QTOF). The polyphenol identification criteria were:
224 retention time, UV-B Vis spectra, MS/MS fragmentation spectra, bibliographical references

225 (Baldi et al., 1995; Cuykens et al., 2004; García Salas et al., 2013) and available database
226 (Massbank).

227 **2.6. Statistical analysis**

228 Data were subjected to analysis of variance (ANOVA) followed by Tukey's test.
229 Differences at $p < 0.05$ were considered significant. Unless indicated, the assays were
230 conducted at least three times with 6 repetitions per replica.

231

232 **3. RESULTS**

233 **3.1. Lemon flavedo extracts differentially affect conidial growth**

234 Flavedo extract of control lemons (CLE) and flavedo extract of UV-B exposed lemons
235 (UVBLE) affected *P. digitatum* growth on PDA plate (**Fig. 1**). Fungal growth was inhibited
236 with 21 mg DW mL^{-1} of CLE and with 15 mg DW mL^{-1} of UVBLE. Sporulation (asterisks
237 in **Fig.1**) was inhibited with 13.5 and 0.75 mg DW mL^{-1} of CLE and UVBLE, respectively.
238 The time course of conidial germination incubated in fresh PD medium was evaluated after
239 treatments with 15 mg DW mL^{-1} flavedo extracts for different periods of time (**Fig. 2**). Data
240 in **Fig. 2A** show that conidial germination was delayed after treatment for 6 to 24 h with
241 CLE in respect to conidia incubated without extract, reaching 100% of germination at 36 h
242 and 12 h of incubation in fresh medium, respectively. Similar germination delay was
243 achieved after 0.5 h-treatment with UVBLE (**Fig. 2B**). Only 10% of conidia germinated
244 after treatments of 3 and 6 h with UVBLE, meanwhile a total inhibition of germination was
245 obtained after 12 and 24 h of incubation.

246 **3.2. Lemon flavedo extracts differentially alter biomarkers of cellular stress in conidia**

247 Several biomarkers of cellular stress were investigated in conidia suspensions after
248 treatments with 15 mg DW mL^{-1} flavedo extracts (**Fig. 3**). ROS production reached
249 maximal values after UVBLE treatment for just 0.5 h; meanwhile it progressively increased
250 with CLE treatment, with values at 24 h of about half to those reached with UVBLE
251 treatment (**Fig. 3A**). With both extracts, TBARS production increased progressively as a
252 function of time, being significantly higher with UVBLE than with CLE (**Fig. 3B**).
253 Conidial oxygen consumption after CLE treatment underwent a slight decrease, while it
254 was severely affected after UVBLE treatment of just 3 h (**Fig. 3C**). Membrane
255 permeability, measured as fluorescent conidia, increased progressively as a function of time
256 (**Fig. 3D**). Conidia treated for 6 h with UVBLE or CLE, showed around 90 or 10% of
257 fluorescent cells, respectively. In the latter condition, only 50% of cells presented
258 membrane damage after 24 h of treatment.

259 **3.3. Chemical and biological characterization of flavedo extracts**

260 Flavedo extracts from geo-referenced lemon samples were used for phytochemical analysis.
261 **Fig. 4** shows the chromatograms from single lemon extracts (CLE and UVBLE), which are
262 representative of twelve different assays. From the comparison of each set of
263 chromatograms, it was possible to detect two peaks (A and B eluting at 32.8 min and 35.9
264 min, respectively) with stable behavior in response to irradiation (**Fig. 4 A** and **B**). In all
265 cases, UV-B treatment decreased peak A and increased peak B (**Fig. 4C**). Fractions
266 corresponding to peaks A and B were further analyzed for antioxidant and antifungal
267 activities. The 50% of $ABTS^{\bullet+}$ was discolored with a Fraction B concentration of around
268 0.5 µg GAE mL^{-1}, while a tenfold higher concentration of Fraction A was necessary to
269 attain the same antioxidant capacity. As shown in **Fig. 5**, both fractions were able to inhibit

270 *P. digitatum* growth; however, the minimal concentration needed to inactivate *P. digitatum*
271 was fivefold lower in Fraction B in respect to Fraction A (10 and 50 mg FWmL^{-1},
272 respectively).
273 The determination of the polyphenol composition of Fractions A and B showed that both
274 samples were complex (**Table 1**). Luteolin, apigenin and diosmetin were detected in both
275 fractions; however, the relative concentrations of these flavones were different. Luteolin
276 content increased and apigenin content diminished in Fraction B in respect to Fraction A. In
277 Fraction B, the glycosylated apigenin or vitexin (apigenin-8-C-hexose), the glycosylated
278 diosmetin (diosmetin-8-C-glucoside), the flavanona naringenin and the flavanol limocitrin
279 were also found.
280

281 **4. DISCUSSION**

282 The application on lemons of a short-term dose of UVBR increased the total phenolic
283 compounds and flavonoid contents in flavedo, contributing to prevent green mold in
284 postharvest lemons (Ruiz et al., 2016). In this work it was demonstrated that lemon flavedo
285 extracts have *in vitro* antifungal activity against *P. digitatum*, which was intensified in
286 extracts of UVBR exposed fruit. Germination and different physiological parameters of
287 conidia were more affected after treatment with UVBLE than with CLE. By HPLC
288 analysis, several compounds were identified that could be responsible for the enhancement
289 in antimicrobial activity of the irradiated lemon extracts.
290 Flavedo extracts inhibited mycelial growth and sporulation in a dose dependent manner,
291 showing differences of both extracts in the higher concentrations assayed. Inhibition of
292 conidial germination and increased of TBARS, ROS and membrane permeability were

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293 detected after treatments with UVBLE; while exposure to CLE produced delayed in the
294 germination and low values of oxidative stress biomarkers. This fact could be attributed to
295 the capacity of cells to trigger repair or adaptation mechanisms after mild treatments. In
296 correlation, conidial respiratory activity was only decreased after exposure to UVBLE,
297 indicating an alteration of mitochondrial function. It is possible to associate the low
298 respiratory rates or zero respiration to the inhibition of germination, since cell division
299 depends on energy. The decrease of respiration can be also associated with the high ROS
300 production, showing an inversely proportional relation between these parameters, in
301 agreement with other reports (Allen et al., 2010; Gyulkhandanyan et al., 2003; Monteiro et
302 al., 2004).
303 It has been reported that antimicrobial activity of citrus fruit peel against *Penicillium* spp.
304 can be enhanced applying physical treatments. Such are the results of the application of
305 heat on the orange *Citrus sinensis* (Ballester et al., 2013), UV-C radiation on *Citrus*
306 *sinensis* (Rodov et al., 1992) and *Citrus aurantium* oranges (Arcas et al., 2000), and the
307 application of UV-B light on *Citrus unshiu* (cv. Aoshima unshu) mandarin (Yamaga et al.,
308 2016). These studies carried out on oranges inferred that UV-C radiation and heat would
309 act as inducer mechanisms of phenolic compounds synthesis, increasing their pathogen
310 resistance. In our work, quantitative changes of flavonoids content in UVBLE in respect to
311 CLE were seen in the two close chromatographic Fractions A and B. In fraction B, a
312 significant accumulation of luteolin and a decrease of apigenin concentration were detected.
313 Besides, the glycosylflavones (vitexin and diosmetin-8-glucoside), the flavanone
314 (naringenin) and the flavonol (limocitrin) were only detected in Fraction B. The presence of
315 flavonol glycosides in UVBR exposed samples is in agreement with the reports about peach

316 and nectarine fruit (Scattino et al., 2014) and *Kalanchoe pinnata* (dos Santos Nascimento et
317 al., 2015), indicating that UVBR is an effective tool to modulate the concentration of
318 health-promoting compounds, mainly of polyphenols. For instance, naringenin has
319 antifungal activity against *P. digitatum* as reported by Ortuño et al. (2006), luteolin has
320 antifungal activity against *Alternaria alternata* (Chiruvella et al., 2007), and vitexin has
321 antibacterial capacity (Basile et al., 1999). Fraction B had higher antifungal activity than
322 Fraction A may be by the differential presence of phenolic compounds. The mechanism by
323 which phenolic compounds act as antimicrobial agents seems to be related to their
324 structures. The presence of at least a free hydroxyl group should be linked to the mentioned
325 activity (Mori et al., 1987), since hydroxyls may act by uncoupling oxidative
326 phosphorylation (Parvez et al., 2004; Tomás Barberán et al., 1990; Wang et al., 1989). The
327 glycosylflavones found in Fraction B present a higher number of free hydroxyl groups than
328 aglycones flavones of Fraction A; hence, it could partially argue the higher antimicrobial
329 capacity in UVBLE than in CLE found in this study.
330 In conclusion, our approach is a first step to understand the mechanisms acting against *P.*
331 *digitatum* on lemon peel. The identification of some phenolic compounds in a flavedo
332 fraction that is enriched after the short-time exposure of lemons to UVBR provides new
333 knowledge about the possible molecules responsible for the control of the pathogen, which
334 can be elicited by physical methods.
335

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342

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534 **Figure captions**

535 **Fig.1.** Effect of increasing concentrations of flavedo extracts of control lemons (CLE) and
536 UV-B exposed lemons (UVBLE) on *P. digitatum* growth. Colony diameters were measured
537 after 5 d of incubation. Control conidia (-) were grown on PDA without extracts. Asterisks
538 indicate the absence of sporulation. Different letters indicate statistical differences
539 according to Tukey's test ($p > 0.05$).

540 **Fig.2.** Effect of increasing exposure time to flavedo extracts of control lemons (CLE) and
541 UV-B exposed lemons (UVBLE) on *P. digitatum* conidia germination. Conidia were grown
542 on PD without extracts (-).

543 **Fig.3.** Biomarkers of cellular stress in *P. digitatum* conidia. **A:** ROS production; **B:**
544 TBARS production; **C:** oxygen consumption; **D:** membrane permeability. In all cases, the
545 effect of increasing exposure time to flavedo extracts of control lemons (CLE) and UV-B
546 exposed lemons (UVBLE) was shown. Conidia incubated without extracts (-). Different
547 letters indicate statistical differences according to Tukey's test ($p > 0.05$).

548 **Fig.4.** Chromatographic profiles of flavedo extracts. **A:** flavedo extracts of control lemons
549 (CLE); **B:** flavedo extract of UV-B exposure lemons (UVBLE). Data are representative of
550 12 HPLC independent runs. **C:** Concentration of Fraction A and Fraction B in CLE and
551 UVBLE. Data are means of the 12 determinations. Different letters indicate statistical
552 differences according to Tukey's test ($p > 0.05$).

553 **Fig.5.** Antifungal activity of Fractions A and B against *P. digitatum*. Conidial supensions
554 were exposed for 12 h to increasing concentrations of Fractions A or B, as indicated in the
555 picture. After 5 d of incubation, colony diameters (mm) were measured, as depicted in the
556 Table. Experiments were carried out by triplicate with four repetitions per replication.

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557 Different letters in Table indicate statistical differences according to Tukey's test ($p >$
558 0.05).

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Table1. Compounds of lemon extracts indentified in Fractions A and B by HPLC/MS polyphenol analysis

Fraction	Retention time (RT)	Molecular Ion (m/z)	Fragmentation MS/MS (m/z)	Tentative Identification	Family	Concentration (µg/g)
A	25.1	285	267	Luteolin	flavone	0.14
	28.3	269	225	Apigenin	flavone	1.43
	28.5	299	284	Diosmetin	flavone	0.55
B	24.9	285	267	Luteolin	flavone	0.29
	28.1	269	225	Apigenin	flavone	0.64
	28.3	299	284,255	Diosmetin	flavone	0.53
	16.6	431	311, 341	Vitexin (apigenin-8-C-hexose)	flavone	0.40
	17.5	461	341, 371	Diosmetin-8-C-glucoside	flavone	1.22
	28.1	345	330, 315	Limocitrin	flavonol	0.97
	26.1	271	253, 197	Naringenin	flavanone	1.61

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Fig. 1

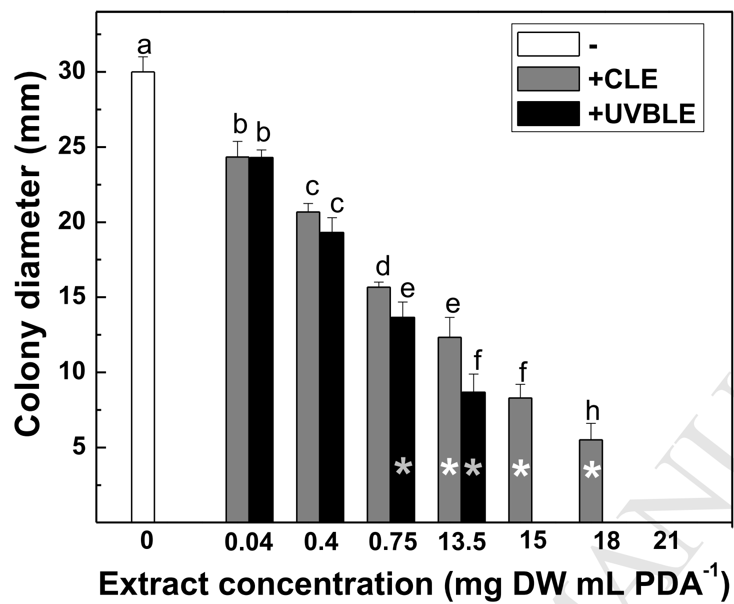

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Fig. 2

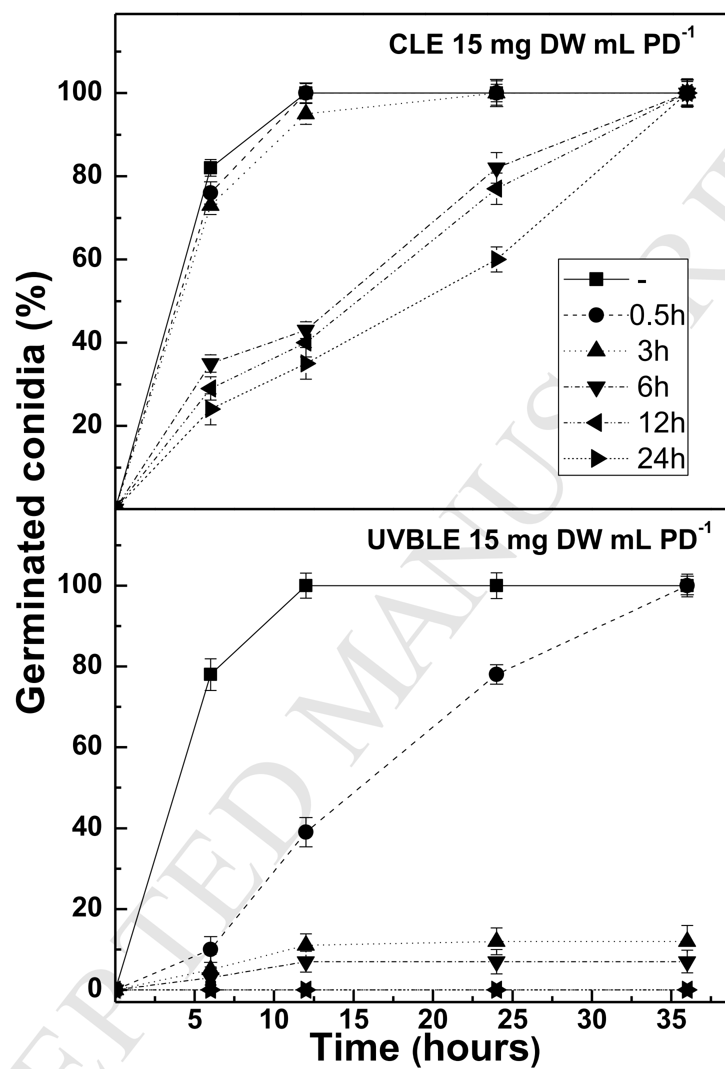

Fig. 3

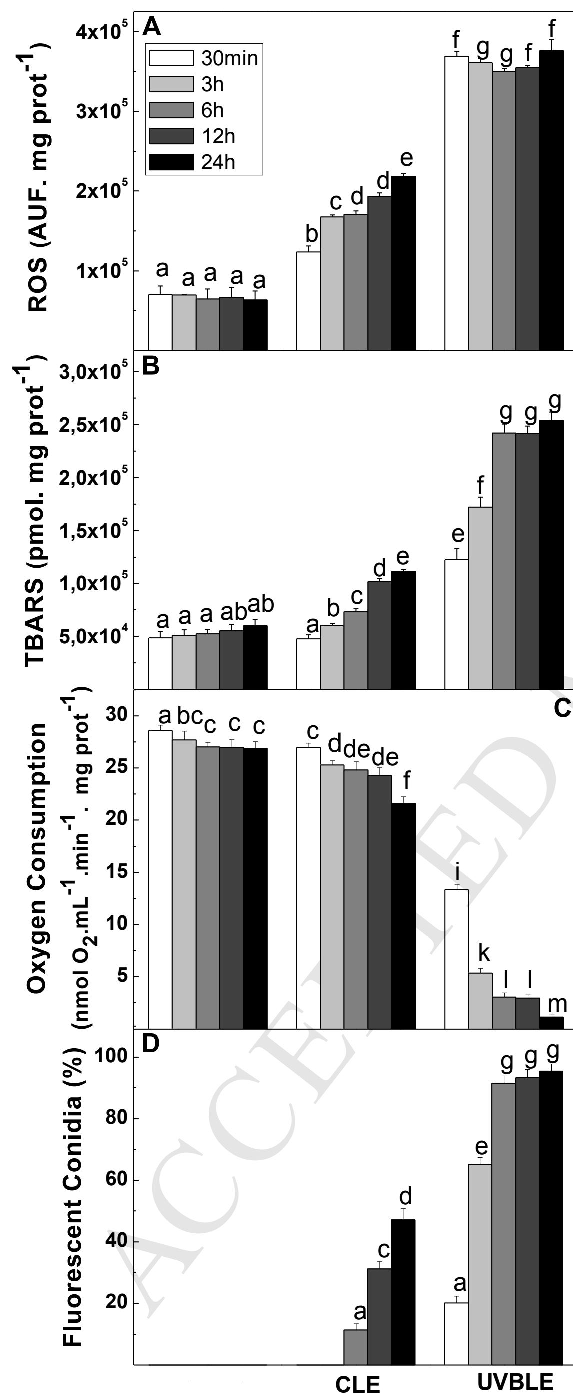

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Fig. 4

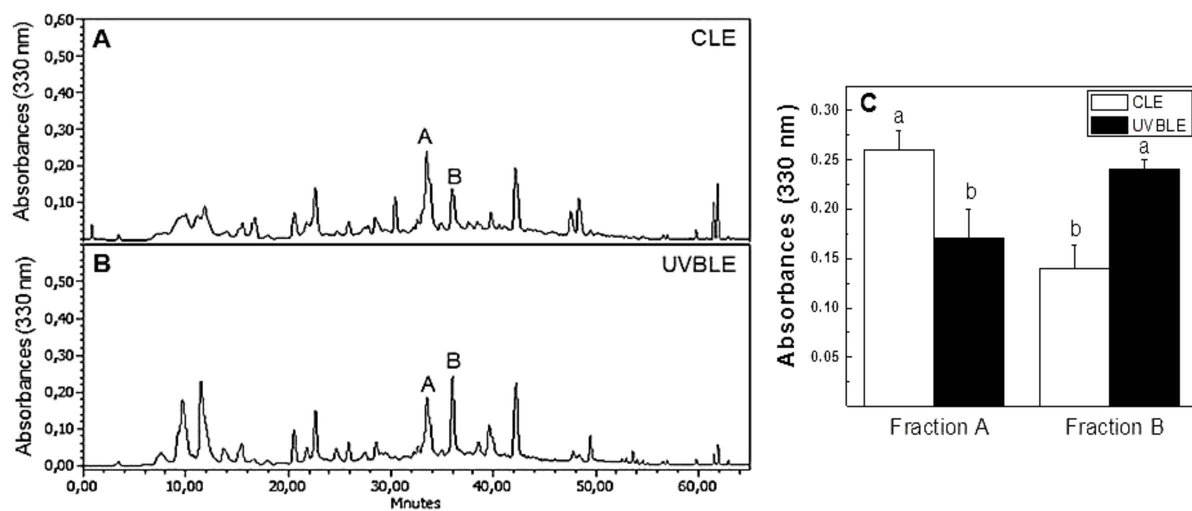

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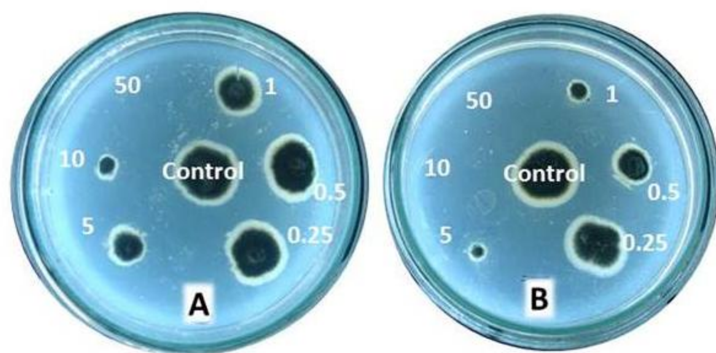
Fig. 5



Concentration (mg mL^{-1})	Fraction A	Fraction B
0	20±2[a]	21±3[a]
0.25	19±3[a]	20±2[a]
0.5	18±1[a]	13±1[b]
1	15±1[b]	7±2[c]
5	11±3[b]	4±2[c]
10	6±1[c]	-
50	-	-

Highlights

- Antifungal activity of lemon peel extract is enhanced by fruit exposure to UVB light
- *P. digitatum* treated with flavedo extract of exposed lemons show severe cell damage
- Phenolic compounds may be the responsible molecules for the pathogen inhibition

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