AN INNOVATIVE ACTIVE CARDBOARD BOX FOR BULK PACKAGING OF FRESH BELL PEPPER

Laura Buendía−Moreno, Sonia Soto−Jover, María Ros−Chumillas, Vera Antolinos−López, Laura Navarro−Segura, María José Sánchez−Martínez, Ginés Benito Martínez−Hernández, Antonio López−Gómez*

Food Safety and Refrigeration Engineering Group, Department of Agronomical Engineering, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, 30203, Cartagena, Murcia, Spain.

* To whom correspondence should be addressed: Tel: +34–968–325516; Email: antonio.lopez@upct.es. Website: http://www.upct.es/~ifsagiid/

ABSTRACT

An antimicrobial active packaging based on controlled essential oils (EOs) vapour release was studied to extend the shelf life of fresh bell peppers bulk-packaged with this active box during storage at 8 ºC (90 % relative humidity (RH)). The active packaging consisted of a corrugated cardboard box coated with a water-based acrylic emulsion including a *β*−cyclodextrin (*β*CD) inclusion complex of a EO mix (carvacrol:oregano:cinnamon 70:10:20 *v:v:v*). The EO mix was efficiently encapsulated within the *β*CD inclusion complex by 94 %. Green, red and yellow peppers packaged within the active box showed 1−2 lower log units of enterobacteria than the control (without the active coating) packaging after 11−18 d at 8 ºC (90 % RH). Furthermore, green/red and yellow peppers showed lower (approximately 1 log unit) mould counts than control samples after 6 and

11 d, respectively. The decay incidence of samples was also highly controlled by the active packaging with percentages lower than 5 % after 18 d while control samples showed decay incidences of 10−15 %. The use of this active box did not negatively affect the physicochemical quality of peppers even showing red and green peppers of the active box better firmness than control samples after 18 d. The shelf life of peppers stored with the active box reached 18 d while samples stored with the control box were rejected. Conclusively, this active packaging allowed to extend the shelf life of green, red and yellow peppers for 18 d at 8 ºC and 90 % RH.

Keywords: Active coating; encapsulated essential oils; *β*−cyclodextrin; inclusion complex; quality.

1. INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is an annual herbaceous vegetable crop from the Solanaceae family. Spain is the fifth largest producer of bell pepper in the world (3 % of the world production) with a national production of 1,293,700 t in 2018 (MAPA, 2018). The wide variety of colours, sizes and shapes, together with its characteristic flavour and high nutritional properties, have led to the growing consumer interest in this vegetable (Meir et al., 1995). The production of bell pepper is mainly (85 %) destined to the fresh market consumption and most of it is sold in bulk packaging (Reche-Mármol, 2010). Nevertheless, bell pepper is a vegetable highly susceptible to pathological deterioration, mainly by *Alternaria alternata*, *Botrytis cinerea* and other soft rots of fungal and bacterial origin (Tournas, 2005). In that sense, appropriate postharvest techniques are needed to control the pathological decay in bell pepper to extend its shelf life. The storage conditions recommended for bell pepper are 7.5−8 ºC and 90 % relative humidity (RH) while higher temperatures may increase water loss and shrivel of the product (Cantwell,

2014). Nevertheless, complementary techniques are needed to control the microbial growth of the product during storage, meeting at the same time the actual consumer interest in natural treatments free from additives.

Essential oils (EOs) are natural substances obtained from plant material (flowers, leaves, seeds, buds, twigs, bark, herbs, wood, fruits and roots), which show excellent *in vitro* antimicrobial properties. Carvacrol, the major component of oregano EO, and the oregano−cinnamon EO combination have shown excellent antimicrobial activities against a wide variety of microorganisms including moulds (Burt, 2004). Due to the hydrophobic nature of EOs, their use is highly limited in water−based treatments of fruit and vegetables. Therefore, the needed EO concentrations to achieve effective antimicrobial effects need to be highly increased when applied in *in vivo* conditions. Nevertheless, high EO concentrations lead to off−flavours typical from EOs being such products rejected by the consumer. The entrapment of EOs by nanoencapsulation has been widely studied in the last decades to increase the EO efficiency through a controlled EO release that limits the microbial growth of food products during storage (Marques et al., 2019). Cyclodextrins (CDs) are cyclic oligomers of *α*−D−glucopyranose. CDs have been intensively studied in food science for several purposes such as the controlled release of antimicrobials, protection of nutritional/health−promoting compounds, stabilization of flavours, elimination of undesired tastes and browning reactions, among other applications (Marques et al., 2019; Pothakamury and Barbosa-Cánovas, 1995; Seglie et al., 2012). The most important CDs at industrial level are *α*− and *β*CD, being the latter one highly extended due to its low cost. *β*CD molecule is made up of 7 D−glucose monomers linked by α (1,4) bonds, exhibiting the shape of a truncated hollow cone. *β*CD is approved as a food additive in Europe (E459), USA and Japan, being established an acceptable daily *β*CD intake of 5 mg kg^{-1} day^{-1} (Mortensen et al., 2016). The *β*CD

77 cavities are hydrophobic, whereas the external faces are hydrophilic. In that sense, the EO
78 entrapment within an *β*CD inclusion complex lets to the increment of the EO stability by
79 reduction of the EO volatility and preservation of their biological properties, while a
80 controlled EO release is allowed (Ayala-Zavala et al., 2008; Marques et al., 2019).
81 Active antimicrobial packaging is an emerging technology that allows to extend the food
82 shelf life through a controlled release of the encapsulated antimicrobial compounds
83 (Khaneghah et al., 2018). Corrugated cardboard is widely used in the European Union as
84 a packaging material for bulk packaging of fresh fruit and vegetables. Cardboard is
85 frequently coated with waterproof lacquers to reinforce its mechanical properties since
86 fruit and vegetables need to be stored at high RH to minimise the water loss during
87 storage. In that sense, active cardboard boxes coated with lacquers including
88 nanoencapsulated antimicrobial compounds may highly extend the shelf life of fresh fruit
89 and vegetables.
90 The objective of this work was to analyse the effect of an innovative active (antimicrobial)
91 cardboard box coated with encapsulated EOs (carvacrol:oregano EO:cinnamon EO mix)
92 (named EOs−*β*CD inclusion complex) on the microbial, physicochemical and sensory
93 quality of fresh bell peppers (green, red and yellow) bulk−packaged with this packaging
94 and stored at 8 ºC (90 % RH). Previously, the used EO mix for the EOs−*β*CD inclusion
95 complex was selected based on their *in vitro* antimicrobial activities against common
96 moulds in bell peppers and pathogens. Furthermore, the active cardboard box was also
97 characterized (*in vitro* antimicrobial activity and physical/mechanical properties) and the
98 EO residues from the active box in peppers were monitored during the product storage.
99

100 **2. MATERIALS AND METHODS**

101 **2.1. Materials**

Carvacrol, oregano EO and cinnamon EO were obtained from Lluch Essence S.L. (Barcelona, Spain). *β*CD was obtained from Roquette (Lestrem, Francia). Waterproof lacquer (a water−based acrylic emulsion named UKAPHOB HR 530; 30 % solids) (authorised for food contact surfaces according to EC (2004)) was acquired from Schill+seilacher GMBH (Böblingen, Germany). Boxes (395×295×210 / 140 / 47 mm) of corrugated cardboard (made with Kraft paper) were manufactured by the company SAECO (Molina de Segura, Spain). All the material for the microbial analyses was acquired from Scharlau Chemie (Barcelona, Spain).

Purified isolates of the following moulds were kindly supplied by J.A. Martínez (Department of Agricultural Engineering, Universidad Politécnica de Cartagena, Spain): *B. cinerea*, *A. alternata, Penicillium* spp., *Aspergillus niger* and *Fusarium* spp. Furthermore, decayed bell peppers with visual mycelial growth were also taken from Fruca company (Balsapintada, Spain) to prepare a mould cocktail. For pathogens, *Escherichia coli* (CECT 7619), *Listeria monocytogenes* LM88, *Salmonella enterica* subsp. *enterica* (CECT 4300) (stored at -80 ºC in glycerine:water solution 1:3 *volume:volume* (*v:v*)) were kindly supplied by A. Palop (Department of Agricultural Engineering, Universidad Politécnica de Cartagena, Spain).

Bell peppers (*Capsicum annuum* L.) (green, red and yellow) were grown in the Southeast of Spain by the company Fruca (Balsapintada, Spain). Peppers were grown under greenhouse conditions according to integrated pest management cultural practices. Peppers were hand−harvested in May 2018 and transported to Fruca installations where they were packaged under the different packaging treatments (control and active boxes) and stored in the cold rooms of this company at 8 ºC (90 % RH).

2.2. *In vitro* antimicrobial effect of EOs

The antimicrobial activities of the EOs (carvacrol, oregano EO and cinnamon EO; either single or different EO mixes), EOs−*β*CD inclusion complexes (prepared using different EO mixes as described in the following section) and the active packaging (including the selected EOs−*β*CD inclusion complex as described in the following sections) were studied. The studied carvacrol:oregano EO:cinnamon EO mixes were: 80:20:0 (*v:v:v*) (EOs1 mix), 80:0:20 (EOs2 mix), 70:10:20 (EOs3 mix) and 60:20:20 (EOs4 mix). As observed, all these EO mixes included a high carvacrol proportion due to its excellent antimicrobial activity against the studied pathogens to ensure the food safety of the product (EC, 2007). EOs1 and EOs2 mixes were selected to study the oregano EO and cinnamon EO contribution to the antimicrobial activity of carvacrol. Meanwhile, EOs3 and EOs4 mixes were selected to study the most appropriate oregano EO proportion to supplement the antimicrobial activity of carvacrol.

Mould and pathogen inocula were prepared as described in the following lines. For moulds, fragments (approximately 3 mm of diameter) of the mould isolates were diluted in Potato Dextrose broth and it was used as the mould inoculum. For pathogens, two subcultures of the frozen pathogen strains were made in nutrient broth at 37 ºC until the stationary phase was reached (after 24 h) at each subculture. The second subculture was selected as the pathogen inoculum. Microbial inocula were adjusted to 4−5 log colony forming units (CFU) mL^{-1} by 10−fold dilution series using buffered peptone water. Then, the adjusted inoculum was spread−plated (0.1 mL) on Petri dishes containing Plate Count Agar.

The antimicrobial activity of EO (single or mixes) were studied by the modified technique ('disc diffusion by vapour contact') of the disc diffusion method (Edwards-Jones et al., 2004). Briefly, EOs were spotted at 300 mg m^{-2} onto 3.5−cm diameter filter paper discs. The EO concentration (300 mg m^{-2}) was selected as the most effective to achieve high

152 antimicrobial activities according to our previous experiments with several EOs (data not
153 shown). Control samples were performed using paper discs without EOs. The
154 EOs−impregnated discs were fixed with sterile adhesive tape (Deltalab; Rubí, Spain) in
155 the centre of the lid of the inoculated Petri dishes. The Petri dishes were then sealed using
156 the sterile adhesive tape. The sealed Petri dishes were face−down incubated at 37 ºC/48
157 h (pathogens) or 25 ºC/7 d (moulds).
158 The antimicrobial activity of the EOs−*β*CD inclusion complexes was also studied with
159 the modified technique ('disc diffusion by vapour contact'). In this case, the prepared
160 EOs−*β*CD inclusion complex powder was manually extended in the centre (on a 2 cm
161 diameter surface) of the lid of the inoculated Petri dishes. The Petri dishes were sealed
162 and stored as described above. Control samples were performed applying only *β*CD
163 powder (without EOs).
164 The antimicrobial activity of the active paperboard (Kraft paperboard) coated with the
165 selected EOs−*β*CD inclusion complex (EOs3 as described in the Results and discussion
166 section)) was also studied with the modified technique ('disc diffusion by vapour
167 contact'). Briefly, Kraft paperboard discs of 6.5 cm diameter were manually coated with
168 the selected EOs−*β*CD inclusion complex at 4 mL m^{-2}. The prepared paper discs were
169 fixed to the lid of the inoculated Petri dishes with the sterile adhesive tape, sealed and
170 incubated as described above. Control samples were performed using Kraft paperboard
171 discs with diluted (according to section 2.3) lacquer (without the EOs−*β*CD inclusion
172 complex).
173 All *in vitro* treatments were performed in triplicate (3 Petri dishes) and microbial counts
174 were expressed as log CFU mL^{-1}. The microbial reductions were expressed as the log
175 unit differences compared to the correspondent control.
176

177 **2.3. Preparation of EOs−*β*CD inclusion complex and characterization**

178 The EO mix selected for the EOs−*β*CD inclusion complex was
179 carvacrol:oregano:cinnamon 70:10:20 *v:v:v* (EOs3 mix) due to its high antimicrobial
180 activity compared to other three EO mixes (EOs1, EOs2 and EOs4 mix) (see Results and
181 discussion section). The EOs3−*β*CD inclusion complex (hereinafter named as "EOs−*β*CD
182 inclusion complex") was prepared using the kneading method (Kamimura et al., 2014;
183 Manolikar and Sawant, 2003). Briefly, 0.15 g of the EOs mix was mixed with 1.14 g of
184 *β*CD in a 1:1 molar ratio in a mortar (including 3 mL of ethanol) and kneaded for 45 min.
185 Then, the obtained EOs−*β*CD inclusion complex was kept for 48 h in a desiccator under
186 vacuum at room temperature and then stored at -20 ºC until its use.

187 The entrapment efficiency (EE) of EOs in the EOs−*β*CD inclusion complex was
188 determined by Differential Scanning Calorimetry (DSC) using a DSC device (model
189 822E, Mettler−Toledo GmbH, Schwerzenbach, Switzerland). Briefly, samples (2 mg) of
190 this inclusion complex were placed in aluminium pans (40 μL). Then, the specimens were
191 heated, under a nitrogen atmosphere (flow rate of 50 mL min^{-1}), from 30 to 400 ºC with
192 a heating rate of 10 ºC min^{-1}. EE was calculated based on an evaporation enthalpy fit of
193 the inclusion complex as described in Eqs. 1 and 2:

194 $$EE = \frac{h_{eEOs}}{h_{EOs}} \times 100 \quad (1)$$

195 $$h_{eEOS} = \frac{h_{complex} \times EOs_{complex}}{100} \quad (2)$$

196 where h_{eEOS} is the enthalpy of the entrapped EOs, h_{EOS} is the enthalpy of the added EOs,
197 $h_{complex}$ is the enthalpy of the EOs−*β*CD inclusion complex and $EOs_{complex}$ is the
198 percentage of added EOs in the EOs−*β*CD inclusion complex.

199 Thermogravimetric/Differential Thermal Analyses (TG/DTA) were conducted to analyse
200 the thermal stability of the EOs−*β*CD inclusion complex. TG/DTA analyses were
201 performed using a TG analyser (model TGA 50, Mettler−Toledo GmbH, Schwerzenbach,

202 Switzerland) in a temperature range from 30 to 600 ºC, a heating rate of 5 ºC min^{-1} and
203 under a nitrogen atmosphere with a flow rate of 50 mL min^{-1}.
204 Fourier Transform Infrared spectroscopy (FTIR) analyses were performed with an FTIR
205 spectrometer (Thermo Scientific Nicolet 5700, Berlin, Germany). Samples (2 mg) were
206 mixed with 200 mg of KBr and this mixture was pressed to form tablets with a thickness
207 of 1 mm. FTIR analyses were conducted in absorbance mode and wavenumbers between
208 400 and 4,000 cm^{-1}.
209 The microscopic morphology of active and control materials from boxes was studied
210 using a scanning electron microscope (SEM) (Hitachi S−3500N SEM). Samples were
211 previously coated with gold in a sputter coater (SC7640, Quorum Technologies, East
212 Sussex, England) and then observed with the SEM at 15 kV.
213
214 **2.4. Preparation of the active box and characterization**
215 The EOs−*β*CD inclusion complex was previously dissolved in water−diluted lacquer
216 (final solid concentration of 8.5 %). The lacquer dilution was made to compensate the
217 addition of the EOs−*β*CD inclusion complex since lacquers with solids > 30 % may
218 difficult their industrial application on the cardboard surface. The lacquer containing the
219 EOs−*β*CD inclusion complex was applied on the cardboard by spraying at industrial scale
220 in the company SAECO (Molina de Segura, Spain). Cardboard coating was made at 4 mL
221 m^{-2} following the manufacturer recommendations to obtain a homogeneous coating on
222 the paperboard surface while reaching adequate waterproof characteristics.
223 The following mechanical properties of boxes were analysed by the Packaging, Transport
224 & Logistics Technological Institute (ITENE; Paterna, Spain): compression resistance
225 (UNE 137001:03), vibration tests at fixed low frequency (PT−04−27), bottom bending

resistance (UNE 49706:02), edgewise crush resistance (UNE EN ISO 3037:13), puncture resistance (UNE ISO 3036:13) and static coefficient of friction (TAPPI T 816om:92).

The water absorptivity and water vapour permeability (WVP) of the paperboard from boxes were also measured in our laboratory. The water absorptivity was determined as previously described (Han et al., 1999; Taboada−Rodríguez et al., 2013). Briefly, paperboard samples were placed on WVP methylacrylate cups (46 mm internal diameter and 27 mm depth) containing distilled water (18 mL). Then, the cups were placed in a forced (speed 3 m s^{-1}) convection chamber at 25 ºC and 50–60 % RH. Cup weights were registered every hour up to 8 h. The water vapour transmission rate (WVTR) was calculated by linear regression of the steady state portion of weight loss *vs* time curve. The WVP (g m m^{-2} s^{-1} Pa^{-1}) was determined by multiplying the WVTR by the thickness (m) of the cardboard and then dividing by the water vapour partial pressure difference between the two sides of the cardboard (Gennadios et al., 1994).

Morphology of the box surface was also studied by SEM. Box samples for SEM analyses were previously coated with gold as described above, and then observed at 15 kV with the SEM.

2.5. Quality of fresh bell peppers bulk-packaged in the active box during storage

Packaging of fresh bell peppers and cold storage were made in the company Fruca (Balsapintada, Spain). Control (CTRL) (without the selected EOs−*β*CD coating) and active (with the selected EOs−*β*CD coating) boxes were used for bulk−packaging of green, red and yellow bell peppers. Such boxes were manually filled with 24 bell peppers. The boxes including the product were then stored at 8 ºC (90 % RH) up to 18 d. Three replicates (three boxes) per packaging treatment (CTRL or active boxes) and pepper variety (green, red and yellow) were taken at each sampling time (1, 6, 11 and 18 d). A

total of 72 boxes were prepared. Microbial (and decay incidence), physicochemical, firmness and sensory quality analyses were performed each sampling time as described in the following subsections.

2.5.1. Microbial quality and decay incidence

Microbial loads of bell peppers were analysed as previously described (López-Gómez et al., 2019; Martínez-Hernández et al., 2017). Briefly, two peppers were mixed with buffered peptone water (1:2 *weight:volume* (*w:v*)) and then homogenised for 1 h at 120 rpm in an orbital shaker at 4 ºC. Viable counts were based on duplicate counts by 10−fold serial dilutions in buffered peptone water. Then, aliquots (1 mL) of the microbial dilutions were pour−plated in Plate Count Agar and Violet Red Bile Glucose Agar for mesophiles/psychrophiles and enterobacteria, respectively. For moulds, microbial aliquots (0.1 mL) were spread-plated on Rose Bengale Agar. Mesophiles, psychrophiles, enterobacteria and moulds were incubated at 31 ºC/48 h, 4 ºC/7 d, 37 ºC/24 h and 25 ºC/7 d, respectively. Results were expressed as log CFU g^{-1}. Each of the three replicates was analysed in duplicate.

2.5.2. Physicochemical quality and firmness

The juice from bell peppers was obtained with a blender (model MX2050; Braun, Germany). The Soluble solids content (SSC) of the obtained juice was determined with a digital handheld refractometer (Atago N1; Tokyo, Kanto, Japan) at 20 ºC and it was expressed as %. The pH of the juice was measured with a pH−meter (Basic20, Crison; Alella, Cataluña, Spain). The titratable acidity (TA) of the diluted juice (5 mL of juice and 45 mL of distilled water) was determined with an automated titrator (model T50; Metter Toledo; Milan, Italy) with 0.1 N NaOH to pH 8.1 being expressed the TA results

276 as equivalents of citric acid in g kg^{-1}. Each of the three replicates was analysed in
277 duplicate.
278 The firmness of bell peppers was determined with a texturometer (model TA XT Plus;
279 TA Instruments; Surrey, UK). First, pepper strips (1×3 cm) were cut (longitudinal
280 direction) with a sharp knife from two opposite sides of each pepper sample. Then, the
281 strips were allowed to equilibrate at room temperature for 30 min prior to the firmness
282 measurements. The firmness of pepper strips was determined with a compression test
283 using a 4.5−kg load cell and a 4−mm−diameter cylinder stainless probe. Each sample was
284 compressed 8 mm at three equidistant (longitudinal axis) points of each strip using a test
285 speed of 20 mm min^{-1}. The peak force (N) necessary to achieve the target distance was
286 recorded. The firmness was determined in two strips (3 equidistant points per each strip)
287 per each pepper. Five peppers were analysed per each replicate (box).
288
289 *2.5.3. Sensory quality*
290 Sensory analyses were performed according to international standards (ASTM, 1986).
291 Tests were conducted in a standard room (ISO, 2007) equipped with ten individual taste
292 booths. Pepper strips (10×3 cm) were served at room temperature in transparent glass
293 plates coded with three random digit numbers. Still mineral water was used as a palate
294 cleanser. The panel consisted of twelve assessors (six women/six men, aged 22–68 years)
295 who were trained in discriminative quality attributes. Colour, flavour, texture and overall
296 quality were assessed using a 9−point hedonic scale of acceptability (9: excellent; 5: fair,
297 limit of usability (LU); 1: extremely bad).
298
299 **2.6. EO residues in fresh bell peppers bulk-packaged in the active box**

EO residues in peppers were analysed during storage. Carvacrol residues were analysed since carvacrol was the major EO component of the used EO mix. Briefly, pepper strips (13 g) were mixed with 20 mL of hexane, vortex for 1 min and then homogenised for 1 h at 120 rpm in an orbital shaker at 4 ºC. The homogenised mixture was filtrated (0.22−µm syringe filters) and analysed with a gas chromatograph coupled to a mass spectrometer (GC−MS model 6890 (Agilent Technologies; Palo Alto (USA)). Carvacrol separation was achieved on a 30 m×0.25 mm×0.25 µm capillary column (CP8982 VF17ms; Agilent Technologies). The carrier gas was helium with a constant flow of 2.8 mL min^{-1} and pressure of 264.8 kPa. The injection was performed in splitless mode. The oven temperature was held at 50 ºC for 1 min after injection, then programmed to reach 235 ºC after 10 min and held at 235 ºC until 29.5 min. MS was set in electronic impact mode (70 eV) with a mass range of 40–400 amu. Source and MS quad temperatures were 230 and 150 ºC, respectively. Carvacrol peak was identified by its mass spectra compared to data from the NIST05a.L database (National Institute for Standards and Technology). Carvacrol was quantified with a carvacrol standard (Sigma, USA) and expressed in fresh weight basis as mg kg^{-1}. Three peppers were analysed per each replicate (box) every sampling time.

2.7. Statistical analyses

Differences between treatments were tested at a 0.05 level of probability with the R studio software. The effects of variety, treatments and storage time were tested with a three-way (variety, treatment and storage time) analysis of variance, followed by a multiple comparison test (Tukey HSD) to identify the differences between factors. Results are reported as mean values ± standard error.

3. RESULTS AND DISCUSSION

3.1. *In vitro* antimicrobial effect of EOs and the EOs−*β*CD inclusion complex

The antimicrobial activities of carvacrol, oregano EO and cinnamon EO were studied against several pathogens and moulds commonly found in bell peppers (López-Gálvez et al., 1997). Carvacrol was more effective than oregano EO and cinnamon EO against the studied pathogens achieving reductions of 0.8, 0.9 and 1.1 for *E. coli*, *Salmonella* spp. and *L. monocytogenes*, respectively (Table 1). Cinnamon EO and oregano EO showed low antimicrobial activities against such pathogens. Attending to moulds, carvacrol also achieved high microbial inactivations (>3 log units) against *A. alternata* and *Penicillium* spp. Oregano EO was the only studied EO effective against *A. niger* showing a reduction of 1.8 log units. The increment of the antimicrobial activity of EOs when combining a major EO component (e.g. carvacrol) with its respective whole EO (i.e. oregano EO) has been widely reported (Burt, 2004). It has been hypothesized that the minor EOs components are critical for the antimicrobial activity of the whole EOs through a possible synergistic effect or potentiating influence. Interestingly, cinnamon EO was effective against *B. cinerea* and *Fusarium* spp. reaching a high mould inactivation (>3 log units). Our previous studies have also shown that cinnamon EO has a high antimicrobial effect against specific saprophyte moulds of citrus fruit, which are resistant to other EOs such as carvacrol and oregano EO (unpublished data). Consequently, EO mixes may have a broad antimicrobial effect against several microorganisms. In that sense, four EO mixes (EOs1, EOs2, EOs3 and EOs4) including a high carvacrol proportion were studied against a cocktail prepared with saprophyte moulds isolated from bell peppers.

The antimicrobial activities of the four EO mixes against the mould cocktail are shown in Figure 1. Furthermore, the antimicrobial activities of these EOs mixes entrapped in the *β*CD inclusion complex (pure inclusion complex and once it was applied on the active

350 packaging material) were also studied against this mould cocktail (Figure 1). As observed,
351 cinnamon EO presence in the EO mixes was necessary to achieve significant microbial
352 reductions since EOs1 discs did not achieve significant (p>0.05) reductions compared to
353 control samples (Figure 1). Although EOs2, EOs3 and EOs4 discs showed similar
354 (p>0.05) reductions among them, a different behaviour was observed when they were
355 applied within the *β*CD inclusion complexes (either pure complex or once applied on the
356 active packaging material). Particularly, EOs3 showed the highest microbial reductions
357 when it was applied either as the pure complex or on the active packaging material. In
358 that sense, the slow EOs3 release from the *β*CD inclusion complex achieved the best
359 control of studied saprophyte moulds of peppers being this inclusion complex selected
360 for the *in vivo* experiments.
361
362 **3.2. Characterization of the EOs−*β*CD inclusion complex**
363 The selected EOs−*β*CD inclusion complex showed an EE of 94.4 % according to Eqs. 1
364 and 2. Such high EO entrapment within the *β*CD may be owed to the low molecular
365 weight of EOs (Asbahani et al., 2015). The obtained EE of the inclusion complex is in
366 agreement with previous data (up to EE of 99.8 %) using also the kneading method to
367 entrap EOs within *β*CD (Marreto et al., 2008). The latter authors attributed this high EE
368 to the absence of heating steps and long complexation times, typical from other
369 encapsulation methods like the slurry procedure, which may lead to an important EO
370 evaporation.
371 The TG/DTA analyses of the EOs−*β*CD inclusion complex shows data regarding EO
372 entrapment and thermal stability of the inclusion complex when compared to the pure
373 *β*CD (Supplementary material 1). An initial water/EO loss from 100 to 180 ºC was
374 observed for both pure *β*CD and the EOs−*β*CD inclusion complex. *β*CD decomposition

started to occur at 275 ºC as previously reported (Marreto et al., 2008). The EO entrapment within the *β*CD inclusion complex is corroborated by the acute mass loss observed between 180 and 270 ºC, which is attributed to the EO release (Marreto et al., 2008).

The EO entrapment within the *β*CD inclusion complex was further confirmed by DSC thermograms (Supplementary material 2). The pure EOs3 mix showed an endothermic phase starting at 237−240 ºC reaching a peak at 248.3 ºC, which corresponded to the EO boiling point (Asbahani et al., 2015). The observed exothermic peaks from 300 to 340 ºC were possibly owed to melting and thermal decomposition of the *β*CD itself as previously found (Kamimura et al., 2014; Seo et al., 2010). The low negative enthalpy values (140.6 and 291.2 J g^{-1} for the EOs−*β*CD inclusion complex and EOs3, respectively) are typical from low energy interactions like those occurred within the EOs−*β*CD inclusion complex: 1) hydrophobic interactions (resulted from the displacement of water molecules from the *β*CD cavity), 2) increment of van der Walls interactions between the molecules, and 3) formation of hydrogen bonds, among others (Marreto et al., 2008; Mourtzinos et al., 2007).

The interactions between host and guest molecules occurred in the EOs−*β*CD inclusion complex were further studied by FTIR analyses (Supplementary material 3). The pure EOs3 showed characteristic FTIR peaks of its components (Supplementary material 3): carvacrol, oregano EO and cinnamon EO. The typical FTIR absorptions from carvacrol and oregano EO were found at 3,367 (−OH stretch), 2,826−2,959 (C−H stretch), 1,591 (alkene C=C), and 1,400 cm^{-1} (aromatic C=C). Furthermore, observed 1,510 and 1,605 cm^{-1} peaks are characteristic from the cinnamon EO, which correspond to the stretching absorption of benzene ring and the stretching of C=O of the aldehyde group (Munhuweyi et al., 2018). Stretching vibration peaks for the aromatic hydroxyl groups were identified

400 at 1,250 cm^{-1}. Out of plane stretching peaks due to aromatic C–H bonds were observed
401 in the 900−650 cm^{-1} range. The aromatic CC stretching was also elucidated at 800 cm^{-1}.
402 The FTIR spectra of the EOs−*β*CD inclusion complex showed peaks shifts and intensity
403 changes comparing to the FTIR spectra of pure EOs3. The characteristic peaks of *β*CD
404 were identified at 3,300 (−OH stretch), 2,925 (vibration of C−H stretch), 1,643 (bending
405 of H–O–H), 1,157 (vibrations of the asymmetric stretch of the C–O–C) and 1,023 cm^{-1}
406 (symmetric stretching link C–O–C) (Wang et al., 2014) when studying the pure *β*CD
407 (Supplementary material 3). Nevertheless, the FTIR spectra of the EOs−*β*CD inclusion
408 complex showed minimal differences compared with the *β*CD spectra. The latter
409 differences between spectra of pure EOs3, *β*CD and the EOs−*β*CD inclusion complex
410 have been also reported (Marques et al., 2019).
411 The morphology of free (pure) *β*CD and the EOs−*β*CD inclusion complex was studied by
412 SEM (Supplementary material 4). The morphology of *β*CD was not substantially changed
413 after the EO inclusion as observed in the SEM captions. Small particles on crystal surfaces
414 were also observed due to agglomeration processes of other particles as it has been
415 described (Songkro et al., 2012).
416

417 **3.3. Characterization of the active box**

418 The mechanical and hydrophobic properties of the CTRL and the active box including
419 the EOs−*β*CD inclusion complex are shown as Supplementary material 5. The active box
420 showed lower bottom bending and edgewise crush resistances than the CTRL box due to
421 the lacquer application. Nevertheless, such resistance reductions would not compromise
422 the needed box resistance for fruit and vegetable packaging. On the other side, the active
423 box showed higher static coefficients of friction compared to the CTRL box, which is
424 beneficial from the production and logistic view since friction must be adequate. As

425 expected, the active box presented lower water absorptivity and vapour permeabilities
426 than the CTRL box due to the waterproof properties of the used lacquer. SEM captions
427 of boxes (Supplementary material 6) showed that the lacquer application did not alter the
428 morphology or fibre crosslinking of the packaging material. Furthermore, a homogeneous
429 particle distribution of the EOs−*β*CD inclusion complex was observed on the active box
430 surface.
431
432 **3.4. Microbial quality and decay incidence of fresh bell peppers bulk−packaged with**
433 **the active box**
434 The antimicrobial benefits of the controlled EO release from the EOs−*β*CD inclusion
435 complex were observed during the storage of bell peppers packaged with this active box
436 (Table 2). The pepper variety factor showed statistical interactions with the packaging
437 treatment factor on mesophilic, psychrophilic and mould loads (Table 2). In that sense,
438 green and red peppers packaged within the active box showed higher log reductions
439 (compared to the CTRL box) than yellow peppers. This pepper variety effect on the
440 antimicrobial properties of the active box may be owed to the contents of specific
441 phytochemicals from red and green peppers with high antimicrobial properties like
442 ascorbic acid, carotenoids, etc. (Simonne et al., 1997). The highest bacteriostatic effects
443 were observed on red peppers at day 6 being reduced as the storage time increased.
444 Particularly, the triple factor interaction (pepper variety × packaging treatment × storage
445 time) was significant (p<0.05) for psychrophiles showing red peppers from the active box
446 2.2, 1.3 and 0.8 lower log units than CTRL after 6, 11 and 18 d, respectively (Table 2).
447 Furthermore, the maximum bacteriostatic activity of the active box with green peppers at
448 days 6−11 (up to 1.4, 3.1 and 1.3 lower log units than CTRL for mesophiles,
449 psychrophiles and enterobacteria, respectively) was decreased to reductions of 1, 1.2 and

2 log units at day 18, respectively. At day 11, the highest bacteriostatic effect was observed for yellow peppers packaged within the active box with 0.9 and 0.5 lower mesophilic and psychrophilic log units compared to the CTRL box. Attending to moulds, green and red peppers packaged in the active box showed 1 and 0.6 lower log units, respectively, compared to the CTRL box at day 6. Contrary, the antimicrobial effect of the active box on mould loads of yellow peppers was only observed after 18 d with 1 log unit lower compared to CTRL samples. The reduction of such bacteriostatic effect for some microbial groups at the end of storage may be owed to several reasons: 1) reduction of EO content from the inclusion complex (as a result of the controlled release), 2) a possible increasing resistance of the survival bacteria to the studied EO, and 3) nutrient leakage from damaged plant tissues (due to product senescence during storage), which enhances microbial growth masking the bacteriostatic benefits from the active packaging. The decay incidence of samples during storage is shown in Figure 2. As observed, bell peppers stored within the active box showed a lower decay incidence during storage compared to CTRL samples. The decay incidence of samples packaged in the active box was lower than 5 % after 18 d while such incidence raised to 10−15 % in CTRL samples. Due to the observed decay control with the active box, samples were further observed up to 21 d at 8 ºC (data not shown). Decay incidence of samples packaged in the active box was still highly controlled after 21 d with incidences of 2−6 % while such incidence raised to 16−19 % for CTRL samples.

Conclusively, the controlled EO release from the *β*CD inclusion complex led to a bacteriostatic effect clearly observed during storage of bell peppers using the active box. The decay incidence was highly controlled using this active packaging as observed. Fruit and vegetables are usually submitted to temperatures higher than 8 ºC during retail periods (distribution centres, freight, transportation, supermarkets, etc.). At such storage

475 temperatures, higher than the recommended ones, EO release from the inclusion complex
476 may be higher. The latter behaviour has been explained since molecular Brownian motion
477 is accelerated with a rise of temperature that improves the speed of EO releasing from the
478 inclusion complex (Ren et al., 2018). In that sense, the EO release from the active
479 packaging may be increased under abusive storage temperatures leading to a better
480 control of the microbial growth, which is enhanced at such adverse storage temperatures
481 for fruit and vegetables. In that sense, further studies are needed to test the antimicrobial
482 activity of this active packaging at different storage temperatures.
483
484 **3.5. Physicochemical and sensory quality of fresh bell peppers bulk−packaged with**
485 **the active box**
486 The pepper variety factor showed a significant effect on the studied physiochemical
487 parameters (Table 3). As expected, red peppers showed initial SSC and TA higher than
488 green and yellow peppers due to sugar and organic acid biosynthesis during pepper
489 ripening. Sugar biosynthesis during ripening in yellow peppers is lower than red peppers
490 as previously reported (Tsegay et al., 2013), and hereby observed (Table 3). The organic
491 acid biosynthesis during colour turning of pepper ripening was also observed from the
492 higher pH of red and yellow peppers compared to green pepper. TA and SSC of red and
493 yellow peppers increased during storage. Organic acid biosynthesis using sugars as the
494 energy pool is expected during senescence processes of fruit and vegetables.
495 Nevertheless, the observed SSC increment during storage may be explained by the
496 product dehydration (visually not observed) and increase of the activity of hydrolytic
497 enzymes. The firmness of samples decreased during storage due to cell wall softening
498 caused by softening enzymes like pectin methylesterase as it has been reported (Goulao
499 et al., 2010; Rao et al., 2011). In general, packaging of peppers with the active box did

not induce high SSC (changes < 0.5 SSC units) and TA differences (changes < 0.05 TA units) comparing to CTRL samples at day 18. Although yellow peppers stored in the active box showed 1 SSC unit lower than CTRL samples at day 18, no flavour differences between these packaging treatments were appreciated by the panel test (Figure 3).

The overall quality of CTRL red and green peppers was below the limit of usability after 18 d being limited the shelf life of these samples to 11 d at 8 ºC (Figure 3). Nevertheless, samples stored within the active box showed overall quality scores over the limit of usability ranging from 7 to 8 after 18 d. Particularly, CTRL green peppers showed the lowest colour score of 3.7 at day 18 while red peppers, regardless of packaging treatment, showed the highest colour scores. Red and green peppers stored within the active box preserved the product firmness (Table 3, Figure 3) better than CTRL samples after 18 d. This higher product firmness using the active box may be explained by the lower metabolism of cell wall carbohydrates occurred during fungal infection of the product as previously reported (Conway, 1987; Serrano et al., 2005). Nevertheless, no high firmness differences between packaging treatments were observed in yellow peppers after 18 d (Table 3, Figure 3). The latter finding may be explained by the lower initial firmness of this pepper variety (Table 3) leading to less appreciable firmness changes during storage. In that sense, the use of the active box would not negatively affect the physicochemical quality and firmness of samples, showing even better sensory scores that may ensure the consumer acceptance of packaged peppers for 18 d at 8 ºC. Nevertheless, peppers packaged with CTRL boxes showed a shelf life of 11 d at 8 ºC.

3.6. EOs residues in fresh bell peppers bulk−packaged with the active box

Carvacrol (the major EOs component of the used EO mix) residues in peppers stored within the active box were below 1 mg L^{-1} during all storage period (data not shown).

525 Such low EO concentrations were not appreciated in the sensory analyses. To the best of
526 our knowledge, there are no concerns related to oral toxicity by carvacrol in humans.
527 Nevertheless, EOs rich in carvacrol should be used with caution with anticoagulant drugs
528 or other bleeding disorders due to the antiplatelet aggregation activity of carvacrol
529 (Tisserand and Young, 2014). On the other hand, a rabbit oral lethal dose of 100 mg kg^{-1}
530 was reported for carvacrol (Opdyke, 1979). Nevertheless, carvacrol content of a pepper
531 portion of 200 g is far from a theoretical extrapolated lethal dose (from rabbit oral lethal
532 dose) of 8 g of carvacrol for a human adult of 80 kg. In that sense, the carvacrol migrations
533 from the active box to the product would not be detected by consumers and would not
534 represent a health hazard.
535

536 **4. CONCLUSIONS**

537 Active packaging is an emerging technology that may extend the shelf life of food, and
538 fruit and vegetables in particular, through a controlled release of antimicrobial
539 compounds. β−cyclodextrins can be used to encapsulate essential oils with a high
540 entrapment efficiency forming inclusion complexes to be incorporated in the active
541 paperboard box coating. The controlled release of essential oils allowed to extend the
542 shelf life of bell peppers for 18 d at 8 ºC. The active box did not negatively affect the
543 physicochemical quality of peppers while firmness and sensory quality were better
544 maintained compared to non-active packaging. Low essential oil concentrations were
545 found in the samples after 18 d being not detected by the sensory analyses. Storage
546 temperatures higher than the recommended ones may lead to a higher release of essential
547 oils from the inclusion complex allowing a higher control of the product microbial growth
548 at such inappropriate storage temperatures. Nevertheless, the latter hypothesis needs to
549 be corroborated in further studies at different temperatures.

ACKNOWLEDGEMENTS

The industrial and laboratory experiments hereby presented have been financed through a 2017−2018 RIS3Mur Project (Call RIS3Mur August 12, 2016, Department of Economic Development, Tourism and Employment, Government of Region of Murcia, Spain), Approval Number 2 / 16SAE00006.

557 **REFERENCES**

558 Asbahani, A. El, Miladi, K., Badri, W., Sala, M., Addi, E.H.A., Casabianca, H.,
559 Mousadik, A. El, Hartmann, D., Jilale, A., Renaud, F.N.R., Elaissari, A., 2015.
560 Essential oils: From extraction to encapsulation. Int. J. Pharm. 483, 220–243.
561 https://doi.org/10.1016/J.IJPHARM.2014.12.069

562 ASTM, 1986. Physical requirement guidelines for sensory evaluation laboratories. ASTM
563 International, Philadelphia PA (USA).

564 Ayala-Zavala, J.F., Soto-Valdez, H., González-León, A., Álvarez-Parrilla, E., Martín-
565 Belloso, O., González-Aguilar, G.A., 2008. Microencapsulation of cinnamon leaf
566 (Cinnamomum zeylanicum) and garlic (Allium sativum) oils in β-cyclodextrin. J.
567 Incl. Phenom. Macrocycl. Chem. 60, 359–368. https://doi.org/10.1007/s10847-007-
568 9385-1

569 Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in
570 foods—a review. Int. J. Food Microbiol. 94, 223–253.
571 https://doi.org/10.1016/J.IJFOODMICRO.2004.03.022

572 Cantwell, M., 2014. Bell pepper: Recommendations for maintaining postharvest quality
573 [WWW Document]. URL
574 http://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Veg
575 etables_English/?uid=5&ds=799

576 Conway, W.S., 1987. Relationship of bound calcium and inoculum concentration to the
577 effect of postharvest calcium treatment on decay of apples by Penicillium expansum.
578 Plant Dis. 71, 78. https://doi.org/10.1094/PD-71-0078

579 EC, 2007. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on
580 microbiological criteria for foodstuffs. Off. J. Eur. Union 32, 12–29.

581 EC, 2004. Commission Regulation Regulation (EU) No 1935/2004 of 14 January 2011

582 on materials and articles intended to come into contact with food and repealing
583 Directives 80/590/EEC and 89/109/EEC. Off. J. Eur. Union 338, 4–17.
584 Edwards-Jones, V., Buck, R., Shawcross, S.G., Dawson, M.M., Dunn, K., 2004. The
585 effect of essential oils on methicillin-resistant Staphylococcus aureus using a
586 dressing model. Burns 30, 772–777. https://doi.org/10.1016/J.BURNS.2004.06.006
587 Gennadios, A., Weller, C.., Gooding, C.., 1994. Measurement errors in water vapor
588 permeability of highly permeable, hydrophilic edible films. J. Food Eng. 21, 395–
589 409. https://doi.org/10.1016/0260-8774(94)90062-0
590 Goulao, L.F., Almeida, D.P.F., Oliveira, C.M., Almeida, D.P.F., Oliveira, C.M., 2010.
591 Effect of enzymatic reactions ontexture of fruits and vegetables, in: Bayindirli, A.
592 (Ed.), Enzymes in Fruit and Vegetable Processing. CRC Press, Boca Raton (USA),
593 pp. 85–136. https://doi.org/10.1201/9781420094343-8
594 Han, J H, Krochta, J M, Han, Jung H, Krochta, John M, 1999. Wetting properties and
595 water vapor permeability of whey-protein-coated paper. Trans. ASAE 42, 1375–
596 1382. https://doi.org/10.13031/2013.13300
597 ISO, 2007. ISO 8589:2007 - Sensory analysis - General guidance for the design of test
598 rooms.
599 Kamimura, J.A., Santos, E.H., Hill, L.E., Gomes, C.L., 2014. Antimicrobial and
600 antioxidant activities of carvacrol microencapsulated in hydroxypropyl-beta-
601 cyclodextrin. LWT - Food Sci. Technol. 57, 701–709.
602 https://doi.org/10.1016/J.LWT.2014.02.014
603 Khaneghah, A.M., Hashemi, S.M.B., Limbo, S., 2018. Antimicrobial agents and
604 packaging systems in antimicrobial active food packaging: An overview of
605 approaches and interactions. Food Bioprod. Process. 111, 1–19.
606 https://doi.org/10.1016/J.FBP.2018.05.001

607 López-Gálvez, G., El-Bassuoni, R., Nie, X., Cantwell, N., 1997. Quality of red and green
608 fresh-cut peppers stored in CAs, in: Gorny, J.R. (Ed.), Fresh-Cut Fruits and
609 Vegetables and MAP. University of Davis, Davis CA (USA), pp. 152–157.
610 López-Gómez, A., Ros-Chumillas, M., Antolinos, V., Buendía-Moreno, L., Navarro-
611 Segura, L., Sánchez-Martínez, M.J., Martínez-Hernández, G.B., Soto-Jover, S.,
612 2019. Fresh culinary herbs decontamination with essential oil vapours applied under
613 vacuum conditions. Postharvest Biol. Technol. 156, 110942.
614 https://doi.org/10.1016/J.POSTHARVBIO.2019.110942
615 Manolikar, M.., Sawant, M.., 2003. Study of solubility of isoproturon by its complexation
616 with β-cyclodextrin. Chemosphere 51, 811–816. https://doi.org/10.1016/S0045-
617 6535(03)00099-7
618 MAPA, 2018. Superficies y producciones de cultivos. Avances [WWW Document]. URL
619 https://www.mapa.gob.es/es/estadistica/temas/estadisticas-
620 agrarias/agricultura/superficies-producciones-anuales-cultivos/ (accessed 4.23.19).
621 Marques, C.S., Carvalho, S.G., Bertoli, L.D., Villanova, J.C.O., Pinheiro, P.F., dos
622 Santos, D.C.M., Yoshida, M.I., de Freitas, J.C.C., Cipriano, D.F., Bernardes, P.C.,
623 2019. β-Cyclodextrin inclusion complexes with essential oils: Obtention,
624 characterization, antimicrobial activity and potential application for food
625 preservative sachets. Food Res. Int. 119, 499–509.
626 https://doi.org/10.1016/j.foodres.2019.01.016
627 Marreto, R.N., Almeida, E.E.C.V., Alves, P.B., Niculau, E.S., Nunes, R.S., Matos,
628 C.R.S., Araújo, A.A.S., 2008. Thermal analysis and gas chromatography coupled
629 mass spectrometry analyses of hydroxypropyl-β-cyclodextrin inclusion complex
630 containing Lippia gracilis essential oil. Thermochim. Acta 475, 53–58.
631 https://doi.org/10.1016/J.TCA.2008.06.015

632 Martínez-Hernández, G.B., Amodio, M.L., Colelli, G., 2017. Carvacrol-loaded chitosan
633 nanoparticles maintain quality of fresh-cut carrots. Innov. Food Sci. Emerg.
634 Technol. 41, 56–63. https://doi.org/10.1016/j.ifset.2017.02.005
635 Meir, S., Rosenberger, I., Aharon, Z., Grinberg, S., Fallik, E., 1995. Improvement of the
636 postharvest keeping quality and colour development of bell pepper (cv. 'Maor') by
637 packaging with polyethylene bags at a reduced temperature. Postharvest Biol.
638 Technol. 5, 303–309. https://doi.org/10.1016/0925-5214(94)00035-Q
639 Mortensen, A., Aguilar, F., Crebelli, R., Di Domenico, A., Dusemund, B., Frutos, M.J.,
640 Galtier, P., Gott, D., Gundert-Remy, U., Leblanc, J., Lindtner, O., Moldeus, P.,
641 Mosesso, P., Parent-Massin, D., Oskarsson, A., Stankovic, I., Waalkens-Berendsen,
642 I., Woutersen, R.A., Wright, M., Younes, M., Boon, P., Chrysafidis, D., Gürtler, R.,
643 Tobback, P., Arcella, D., Rincon, A.M., Lambré, C., 2016. Re-evaluation of
644 β-cyclodextrin (E 459) as a food additive. EFSA J. 14.
645 https://doi.org/10.2903/j.efsa.2016.4628
646 Mourtzinos, I., Salta, F., Yannakopoulou, K., Chiou, A., Karathanos, V.T., 2007.
647 Encapsulation of olive leaf extract in β-cyclodextrin. J. Agric. Food Chem. 55,
648 8088–8094. https://doi.org/10.1021/jf0709698
649 Munhuweyi, K., Caleb, O.J., van Reenen, A.J., Opara, U.L., 2018. Physical and
650 antifungal properties of β-cyclodextrin microcapsules and nanofibre films
651 containing cinnamon and oregano essential oils. LWT 87, 413–422.
652 https://doi.org/10.1016/J.LWT.2017.09.012
653 Opdyke, D.L.J., 1979. Monographs on fragrance raw materials. Food Cosmet. Toxicol.
654 17, 743–745. https://doi.org/10.1016/0015-6264(79)90288-8
655 Pothakamury, U.R., Barbosa-Cánovas, G. V., 1995. Fundamental aspects of controlled
656 release in foods. Trends Food Sci. Technol. 6, 397–406.

https://doi.org/10.1016/S0924-2244(00)89218-3

Rao, T.V.R., Gol, N.B., Shah, K.K., 2011. Effect of postharvest treatments and storage temperatures on the quality and shelf life of sweet pepper (Capsicum annum L.). Sci. Hortic. (Amsterdam). 132, 18–26. https://doi.org/10.1016/J.SCIENTA.2011.09.032

Reche-Mármol, J., 2010. Cultivo del pimiento dulce en invernadero [WWW Document]. URL https://www.juntadeandalucia.es/export/drupaljda/1337160265Cultivo_Pimiento_Invernadero.pdf (accessed 4.23.19).

Ren, X., Yue, S., Xiang, H., Xie, M., 2018. Inclusion complexes of eucalyptus essential oil with β-cyclodextrin: Preparation, characterization and controlled release. J. Porous Mater. 25, 1577–1586. https://doi.org/10.1007/s10934-018-0571-x

Seglie, L., Spadaro, D., Trotta, F., Devecchi, M., Gullino, M.L., Scariot, V., 2012. Use of 1-methylcylopropene in cyclodextrin-based nanosponges to control grey mould caused by Botrytis cinerea on Dianthus caryophyllus cut flowers. Postharvest Biol. Technol. 64, 55–57. https://doi.org/10.1016/j.postharvbio.2011.09.014

Seo, E.-J., Min, S.-G., Choi, M.-J., 2010. Release characteristics of freeze-dried eugenol encapsulated with β -cyclodextrin by molecular inclusion method. J. Microencapsul. 27, 496–505. https://doi.org/10.3109/02652041003681398

Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F., Valero, D., 2005. The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. Innov. Food Sci. Emerg. Technol. 6, 115–123. https://doi.org/10.1016/J.IFSET.2004.09.001

Simonne, A.H., Simonne, E.H., Eitenmiller, R.R., Mills, H.A., Green, N.R., 1997. Ascorbic acid and provitamin a contents in unusually colored bell peppers (Capsicum annuumL.). J. Food Compos. Anal. 10, 299–311.

https://doi.org/10.1006/JFCA.1997.0544

Songkro, S., Hayook, N., Jaisawang, J., Maneenuan, D., Chuchome, T., Kaewnopparat, N., 2012. Investigation of inclusion complexes of citronella oil, citronellal and citronellol with β-cyclodextrin for mosquito repellent. J. Incl. Phenom. Macrocycl. Chem. 72, 339–355. https://doi.org/10.1007/s10847-011-9985-7

Taboada-Rodríguez, A., García-García, I., Cava-Roda, R., López-Gómez, A., Marín-Iniesta, F., 2013. Hydrophobic properties of cardboard coated with polylactic acid and ethylene scavengers. J. Coatings Technol. Res. 10, 749–755. https://doi.org/10.1007/s11998-013-9493-3

Tisserand, R., Young, R., 2014. Constituent profiles, in: Essential Oil Safety. Churchill Livingstone, pp. 483–647. https://doi.org/10.1016/B978-0-443-06241-4.00014-X

Tournas, V.H., 2005. Moulds and yeasts in fresh and minimally processed vegetables, and sprouts. Int. J. Food Microbiol. 99, 71–77. https://doi.org/10.1016/j.ijfoodmicro.2004.08.009

Tsegay, D., Tesfaye, B., Ibrahim, M.A., Yirga, H., Bayleyegn, A., 2013. Cell growth curves for different cell lines and their relationship with biological activities. Int. J. Biotechnol. Mol. Biol. Res. 4, 60–70. https://doi.org/10.5897/IJBMBR2013.0154

Wang, X., Luo, Z., Xiao, Z., 2014. Preparation, characterization, and thermal stability of β-cyclodextrin/soybean lecithin inclusion complex. Carbohydr. Polym. 101, 1027–1032. https://doi.org/10.1016/j.carbpol.2013.10.042

704 **FIGURE AND TABLE CAPTIONS**

705

706 **Table 1.** *In vitro* microbial reductions (log units) of moulds and pathogens with pure EOs
707 (n=3±SD).

708

709 **Table 2.** Microbial loads (log CFU g^{-1}) of fresh bell peppers (green, red and yellow) bulk-
710 packaged with different packaging treatments (AP: active packaging box coated with the
711 EOs−*β*CD inclusion complex; CTRL: active packaging box without the EOs−*β*CD
712 inclusion complex) stored at 8 ºC (n=3±SD).

713

714 **Table 3.** Physicochemical quality (soluble solid content (%), SSC; pH and titratable
715 acidity (mg kg^{-1}), TA) (n=3±SD) and firmness (N) (n=5±SD) of fresh bell peppers (green,
716 red and yellow) bulk-packaged with different packaging treatments (AP: active packaging
717 box coated with the EOs−*β*CD inclusion complex; CTRL: active packaging box without
718 the EOs−*β*CD inclusion complex) stored at 8 ºC.

719

720 **Figure 1.** *In vitro* microbial reductions (log units) against a mould cocktail by disc
721 diffusion (vapour contact variant) using different pure EOs mixes or included in the
722 EOs−*β*CD inclusion complex (pure complex or active material sprayed with the
723 corresponding EOs−*β*CD complex) (n=3±SD). Different letters denote significant
724 differences (p<0.05) among treatments. ns: not significant reductions (p>0.05) compared
725 to control.

726

727 **Figure 2.** Decay incidence (%) of fresh bell peppers (green, red and yellow) bulk-
728 packaged with different packaging treatments (AP: active packaging box coated with the

729 EOs−βCD inclusion complex (bars with points); CTRL: active packaging box without
730 the EOs−βCD inclusion complex (empty bars)) stored at 8 ºC. *denotes significant
731 differences (p<0.05) between AP and CTRL packaging treatments for the same sampling
732 day.
733
734 **Figure 3.** Sensory scores of fresh bell peppers (green, red and yellow) bulk packaged
735 with different packaging treatments (AP: active packaging box coated with the EOs−βCD
736 inclusion complex; CTRL: active packaging box without the EOs−βCD inclusion
737 complex) stored for 11 (A) and 18 d (B) at 8 ºC (n=3).
738

SUPPLEMENTARY MATERIAL

Supplementary material 1. Thermogravimetric/Differential Thermal Analysis (TG/DTA) of *β*CD (A) and EOs−*β*CD inclusion complex (B).

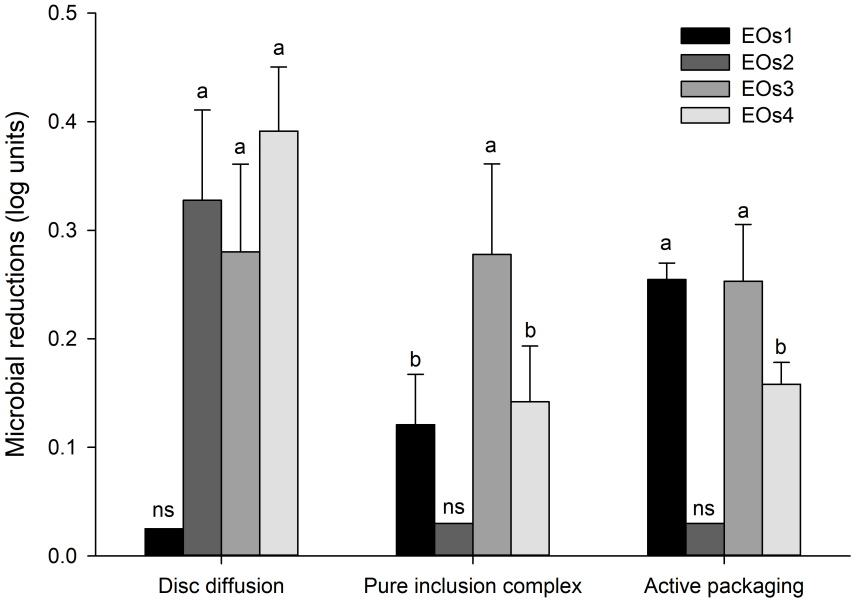
Supplementary material 2. Differential scanning calorimetry (DSC) thermogram of EOs (red line) and EOs−*β*CD inclusion complex (black line).

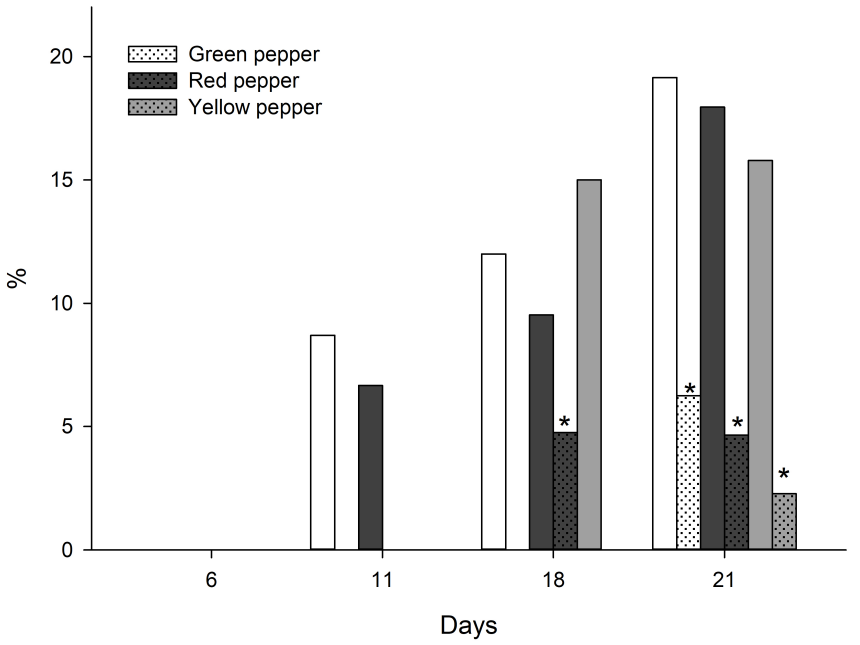
Supplementary material 3. Fourier transform infrared (FTIR) spectra for pure EOs mix (a), *β*−cyclodextrin (b), and EOs−*β*CD inclusion complex (c).

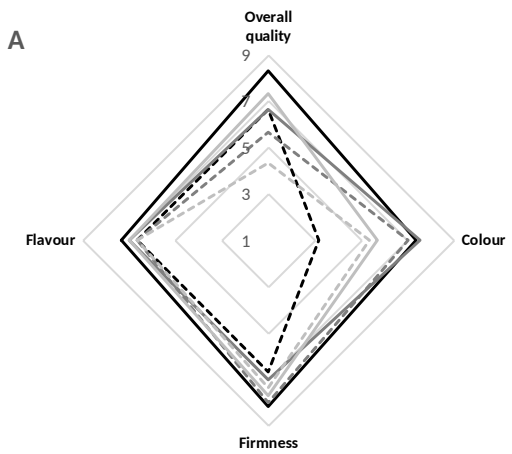
Supplementary material 4. Scanning electron micrographs (SEM) of (a) free *βCD* and (b) EOs−*β*CD inclusion complex.

Supplementary material 5. Mechanical and hydrophobic properties of active (coated with the EOs−*β*CD inclusion complex) and control boxes (without the EOs−*β*CD inclusion complex).

Supplementary material 6. SEM captions of non−sprayed paperboard box (left), control paperboard box (centre) (coated with lacquer without the EOs−*β*CD inclusion complex) and active paperboard box (right) (coated with lacquer with the EOs−*β*CD inclusion complex).


Microbial reductions (log units)
0.5
0.4
0.3
0.2
0.1
0.0
EOs1
EOs2
EOs3
EOs4
a
a
a
ns
b
a
b
ns
a
a
b
ns
Disc diffusion
Pure inclusion complex
Active packaging


Green pepper
Red pepper
Yellow pepper
20
15
10
5
0
%
6
11
18
21
Days
*
*
*
*


A
Overall quality
9
7
5
3
1
Flavour
Colour
Firmness

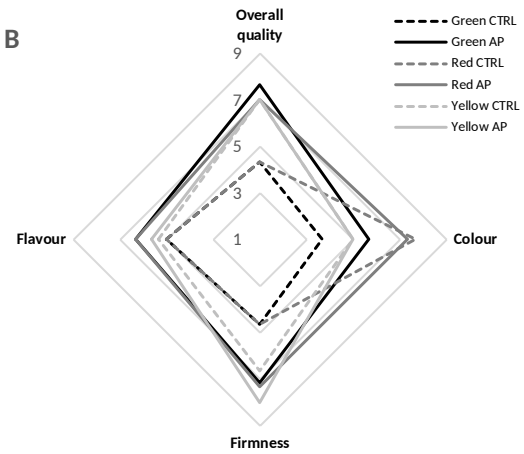

B
Overall quality
9
7
5
3
1
Flavour
Colour
Firmness
Green CTRL
Green AP
Red CTRL
Red AP
Yellow CTRL
Yellow AP

Table 1.

	Carvacrol	Oregano EO	Cinnamon EO
Pathogens			
Escherichia coli	0.78±0.15	0.31±0.06	0.37±0.01
Listeria monocytogenes	1.06±0.28	ns	0.66±0.36
Salmonella spp.	0.86±0.09	ns	ns
Moulds			
Botrytis cinerea	ns	1.41±0.05	+
Alternaria alternata	+	ns	+
Penicillium spp.	+	ns	+
Aspergillus niger	ns	1.79±0.68	ns
Fusarium spp.	ns	ns	+

ns: not significant reductions (p>0.05) compared to control; + reductions higher than 3 log units.

Table 2.

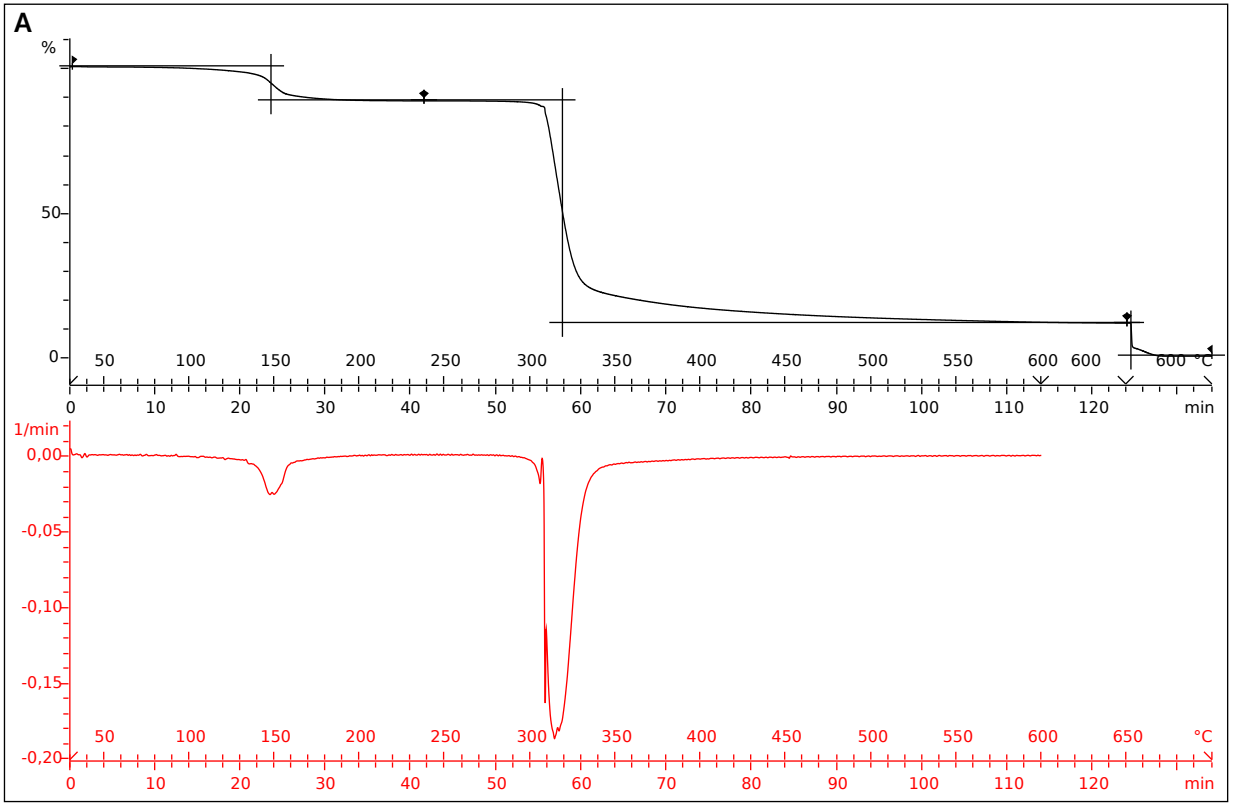
Pepper	Packaging	Storage days	Mesophiles	Psychrophiles	Enterobacteria	Moulds
Green	CTRL	1	4.23±0.22	2.43±0.02	5.28±0.07	1.50±0.71
		6	5.01±0.12	5.64±0.39	5.22±0.21	2.98±0.28
		11	5.80±0.02	6.11±0.80	5.56±0.79	3.43±0.10
		18	6.23±0.12	6.13±0.09	5.62±0.07	4.24±0.29
	AP	1	4.23±0.22	2.43±0.02	5.28±0.07	1.50±0.71
		6	4.31±0.38	3.09±0.13	3.91±0.13	2.00±0.01
		11	4.39±0.55	3.00±0.01	4.54±0.09	2.80±0.28
		18	5.27±0.10	4.95±0.07	3.66±0.51	4.16±0.03
Red	CTRL	1	5.52±0.06	3.27±0.26	5.89±0.01	2.57±0.38
		6	6.46±0.25	6.41±0.02	6.07±0.26	3.21±0.13
		11	6.46±0.38	6.38±0.01	5.96±0.02	3.70±0.10
		18	6.77±0.22	6.06±0.33	6.02±0.03	4.20±0.05
	AP	1	5.52±0.06	3.27±0.26	5.89±0.01	2.57±0.38
		6	4.82±0.04	4.26±0.50	3.52±0.62	2.59±0.16
		11	5.11±0.38	5.06±0.51	4.69±0.62	3.42±0.60
		18	5.48±0.62	5.29±0.63	5.40±0.37	4.20±0.01
Yellow	CTRL	1	5.83±0.14	3.61±0.40	4.21±0.34	2.12±0.16
		6	5.22±0.42	3.61±0.19	4.10±0.02	2.57±0.38
		11	4.50±0.28	3.50±0.71	4.23±0.07	2.65±0.49
		18	5.38±0.60	5.37±0.10	4.42±0.82	4.71±0.11
	AP	1	5.83±0.14	3.61±0.40	4.21±0.34	2.12±0.16
		6	5.08±0.08	4.90±0.05	3.95±0.32	2.59±0.16
		11	3.57±0.38	3.00±0.04	4.16±0.36	3.07±0.84
		18	5.61±0.11	4.94±0.65	3.00±0.05	3.69±0.30
	Variety (A)		(0.31)‡	(0.38)‡	(0.53)‡	(0.28)†
	Packaging treatment (B)		(0.21)‡	(0.26)‡	(0.37)‡	(0.14)*
	Storage time (C)		(0.33)‡	(0.40)‡	(0.31)*	(0.40)‡
	A×B		(0.43)‡	(0.52)‡	ns	ns
	A×C		(0.66)‡	(0.80)‡	ns	ns
	B×C		(0.46)‡	(0.56)‡	(0.44)*	ns
	A×B×C		ns	(1.13)‡	ns	ns

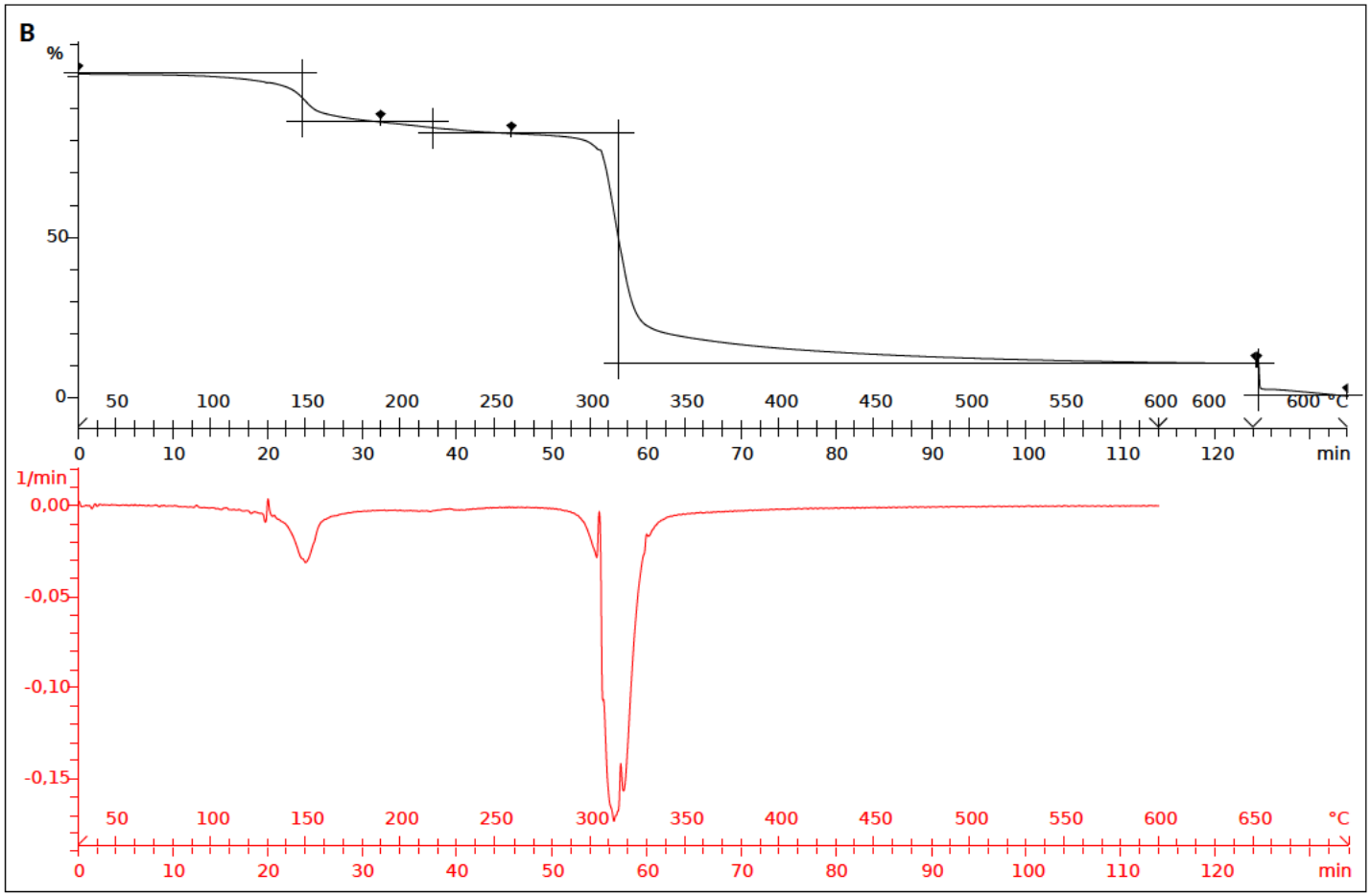
ns: not significant (p>0.05); *, †, ‡ significance for $P \leq 0.05$, 0.01, and 0.001, respectively.

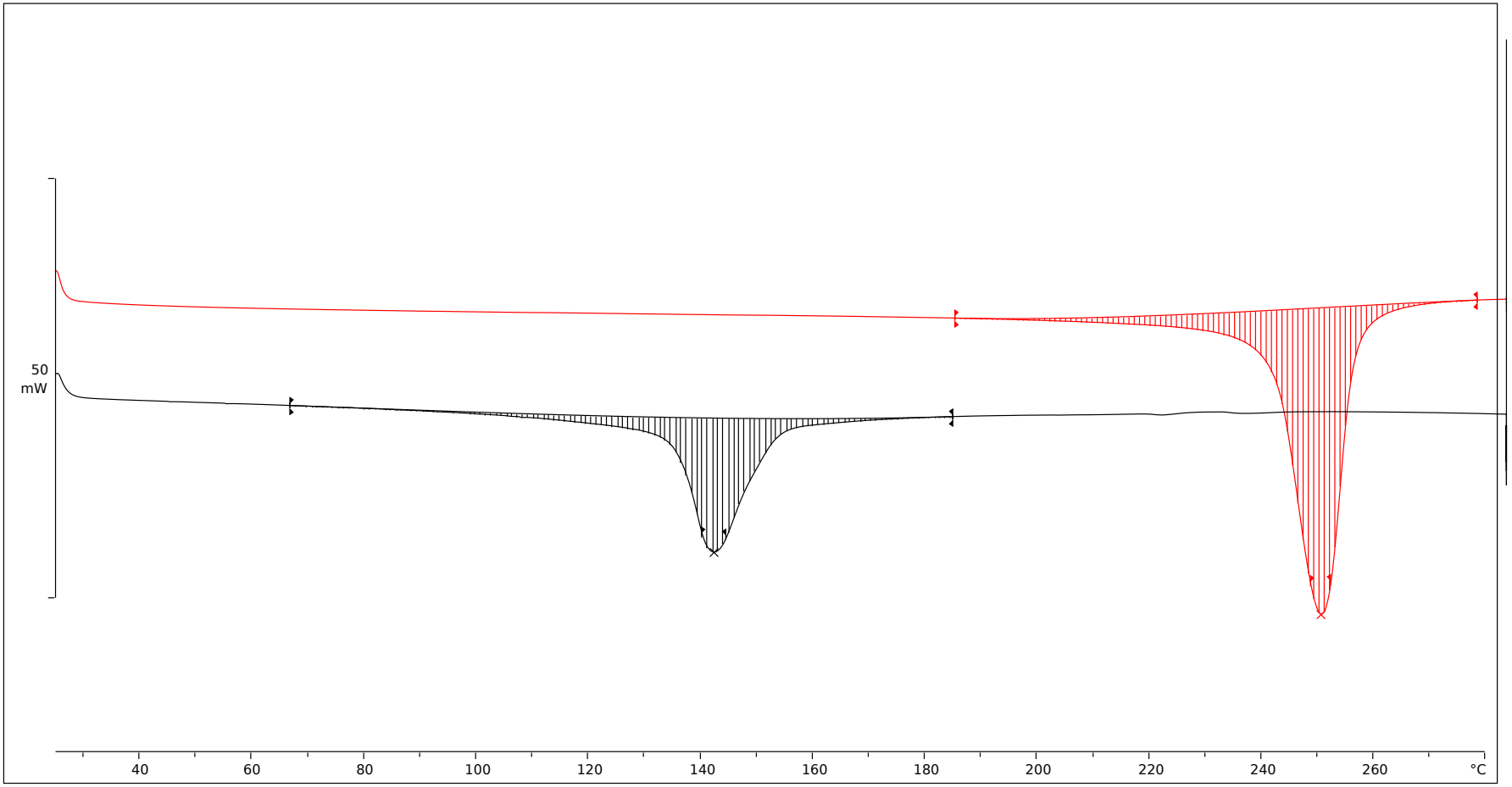
Table 3.

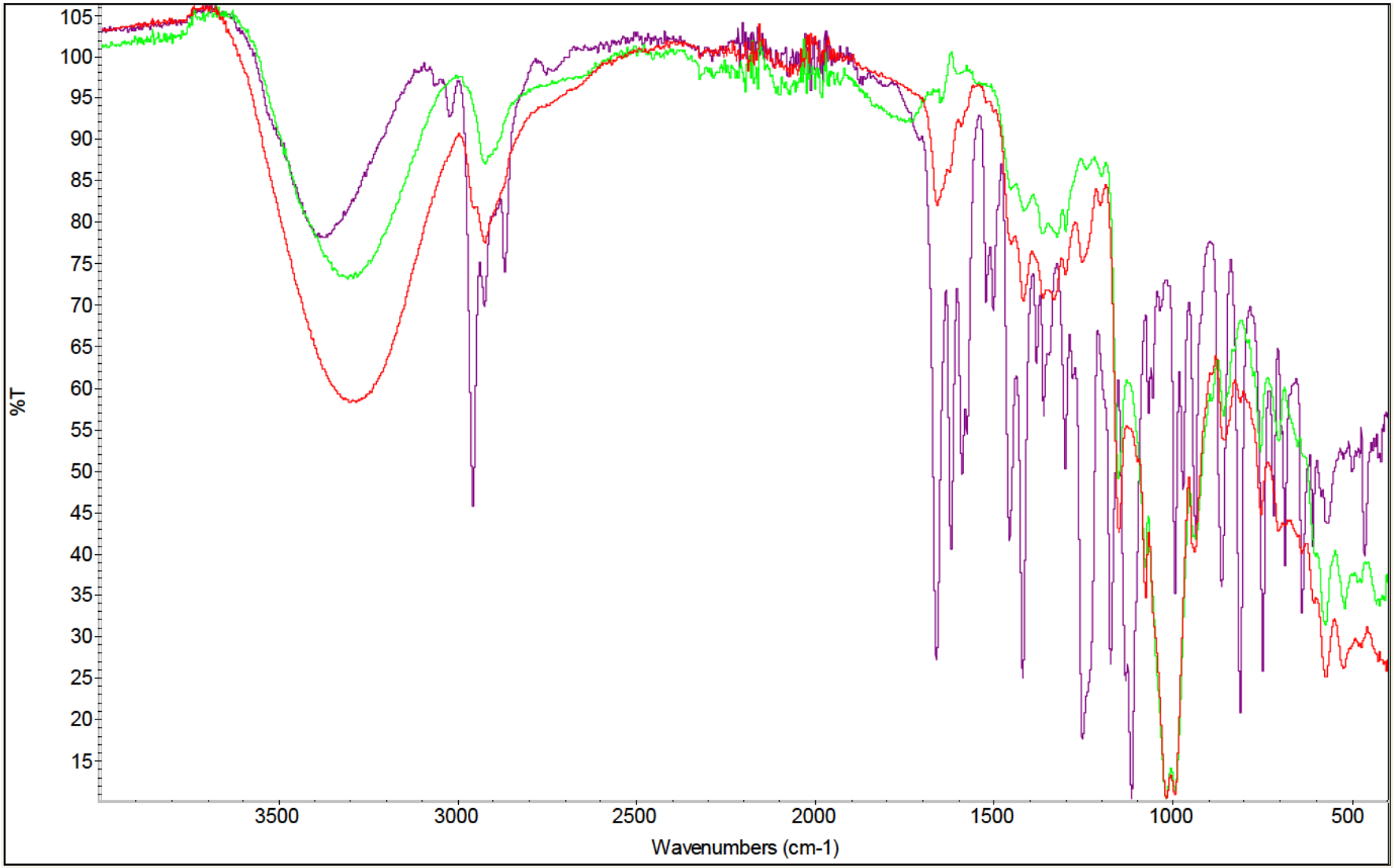
Pepper variety	Packaging	Storage days	SSC	pH	TA	Firmness
Green	CTRL	1	5.0±0.1	6.21±0.01	1.2±0.1	27.1±0.1
		6	5.0±0.2	5.49±0.09	1.2±0.1	27.2±0.1
		11	4.8±0.4	5.27±0.05	1.0±0.1	27.2±1.9
		18	4.8±0.4	5.66±0.03	1.3±0.3	19.5±0.9
	AP	1	5.0±0.1	6.21±0.01	1.2±0.1	27.1±0.1
		6	4.5±0.1	5.67±0.11	0.9±0.1	27.8±1.6
		11	3.8±0.4	5.76±0.06	1.9±0.3	26.0±3.0
		18	5.3±0.4	4.89±0.05	0.9±0.1	22.7±0.8
Red	CTRL	1	6.0±0.2	5.25±0.10	2.6±0.2	19.3±2.1
		6	6.5±0.1	5.04±0.05	2.2±0.1	16.6±2.0
		11	6.8±0.3	4.33±0.16	3.0±0.1	17.0±0.9
		18	7.0±0.2	5.14±0.01	2.3±0.2	15.3±0.8
	AP	1	6.0±0.2	5.25±0.10	2.6±0.2	19.3±2.1
		6	6.3±0.4	5.05±0.04	2.2±0.1	22.3±2.4
		11	6.0±0.2	4.90±0.01	3.0±0.1	20.0±0.8
		18	7.3±0.4	4.64±0.10	2.7±0.2	19.1±0.6
Yellow	CTRL	1	5.2±0.1	5.04±0.06	2.0±0.1	15.9±2.0
		6	6.8±0.3	5.03±0.01	2.0±0.1	16.7±1.1
		11	7.1±0.2	4.52±0.18	2.5±0.1	16.7±0.1
		18	6.0±0.4	4.89±0.08	2.4±0.3	14.3±1.0
	AP	1	5.2±0.1	5.04±0.06	2.0±0.1	15.9±2.0
		6	6.8±0.4	5.04±0.01	2.3±0.1	17.3±1.0
		11	7.0±0.1	4.89±0.01	3.0±0.2	13.4±1.1
		18	5.0±0.4	4.74±0.31	1.9±0.2	12.6±1.5
	Variety (A)		(0.22)‡	(0.10)‡	(0.1)‡	(1.4)‡
	Packaging treatment (B)		(0.15)‡	ns	(0.1)†	(0.5)‡
	Storage time (C)		(0.23)‡	(0.10)‡	(0.2)‡	(1.4)‡
	A×B		ns	ns	ns	(1.4)†
	A×C		(0.46)‡	(0.20)‡	(0.3)‡	(2.9)‡
	B×C		(0.18)*	(0.14)‡	(0.2)‡	ns
	A×B×C		(0.65)‡	(0.21)†	(0.4)‡	ns

ns: not significant (p>0.05); *, †, ‡ significance for $P \leq 0.05$, 0.01, and 0.001, respectively.

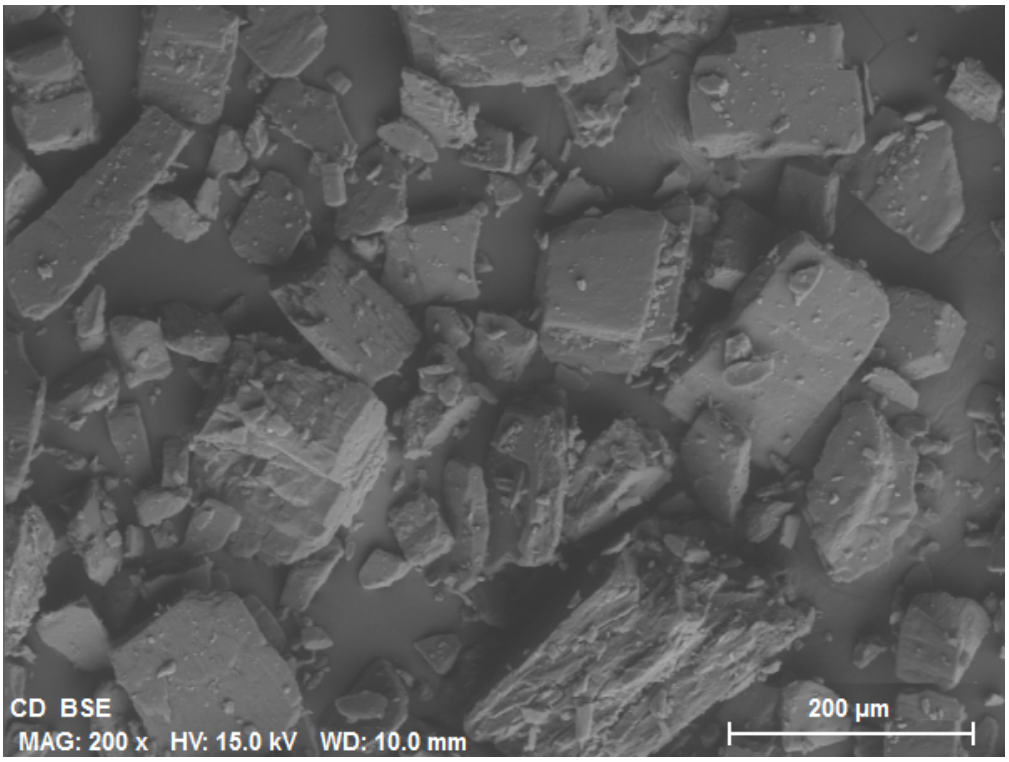

A
%
50
0
50 100 150 200 250 300 350 400 450 500 550 600 600 600 °C
0 10 20 30 40 50 60 70 80 90 100 110 120 min
1/min
0,00
-0,05
-0,10
-0,15
-0,20
50 100 150 200 250 300 350 400 450 500 550 600 650 °C
0 10 20 30 40 50 60 70 80 90 100 110 120 min

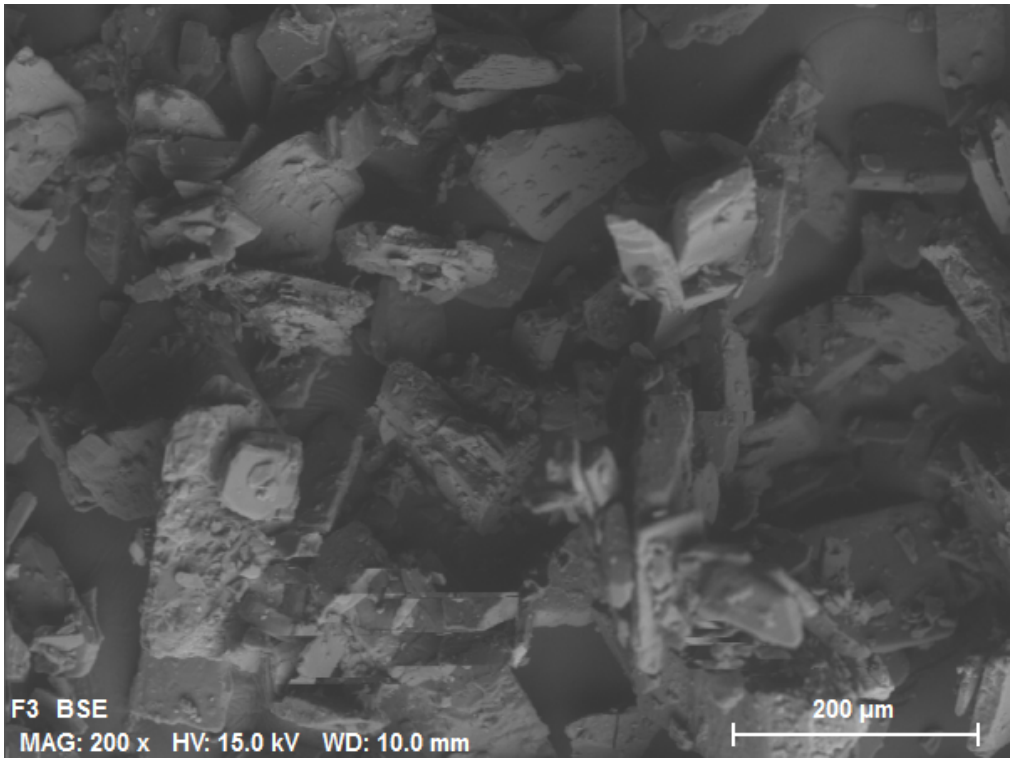

B
%
50
0
50 100 150 200 250 300 350 400 450 500 550 600 600 600 °C
0 10 20 30 40 50 60 70 80 90 100 110 120 min
1/min
0,00
-0,05
-0,10
-0,15
50 100 150 200 250 300 350 400 450 500 550 600 650 °C
0 10 20 30 40 50 60 70 80 90 100 110 120 min


50
mW
40
60
80
100
120
140
160
180
200
220
240
260
°C

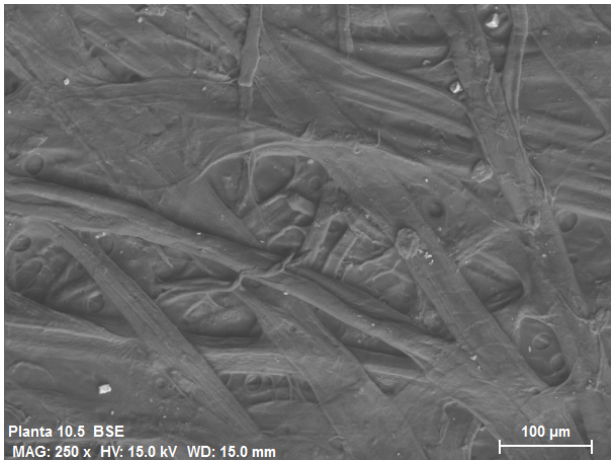


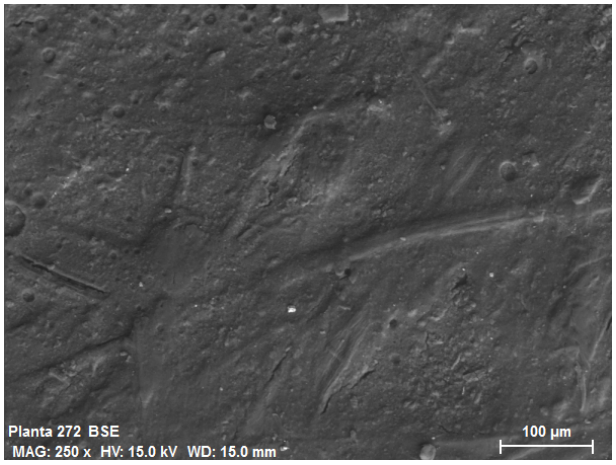
EOS, βCD and CD+F3


CD BSE
MAG: 200 x HV: 15.0 kV WD: 10.0 mm
200 µm


F3 BSE
MAG: 200 x HV: 15.0 kV WD: 10.0 mm
200 µm


Planta_PKV BSE
MAG: 250 x HV: 15.0 kV WD: 15.0 mm
100 µm


Planta 10.5 BSE
MAG: 250 x HV: 15.0 kV WD: 15.0 mm
100 µm


Planta 272 BSE
MAG: 250 x HV: 15.0 kV WD: 15.0 mm
100 µm

Supplementary material 5.

	Control box	Active box
Compression resistance[1]	383.2±39.6 (8.3±1.1)	350.8±34.9 (9.3±0.7)
Vibration at fixed low frequency[2]	Apt	Apt
Bottom bending resistance[3]	4.7±0.2	3.5±0.3*
Edgewise crush resistance[4]	9.0±1.0	6.5±0.7*
Puncture resistance[5]	10.2±0.5	10.1±0.4
Static coefficient of friction[6]	0.42±0.04/0.28±0.05	0.54±0.04*/0.39±0.07*
Moisture content[6]	6.72±0.10	6.95±0.09*
Water absorptivity[7]	109.2±7.1	97.4±4.3*
Water vapor permeability[8]	1.002×10^{-9}	1.395×10^{-10}

[1] expressed in kgf and deformation (between parentheses) in mm; [2] overload of 40 kg; [3] camber in mm; [4] in kN m^{-1}; [5] in J; [6] static coefficient/kinetic coefficient; [7] in g m^{-2}; [8] in g m m^{-2} s^{-1} Pa^{-1}. * denotes significant ($P<0.05$) differences of active box compared to control box.