EFFECT OF FRESH–CUT APPLES FORTIFICATION WITH LYCOPENE MICROSPHERES, REVALORIZED FROM TOMATO BY-PRODUCTS, DURING SHELF LIFE

Ginés Benito Martínez–Hernández, Noelia Castillejo and Francisco Artés–Hernández*

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

* To whom correspondence should be addressed: Tel: +34–968–325509; Email: fr.artes–hdez@upct.es. Website: www.upct.es/gpostref.

http://orcid.org/0000-0002-0689-7301

Abstract

Fresh–cut apple browning has been conventionally tried to control with the help of ascorbic acid dips, although such antioxidant effect is limited. Lycopene, absent in apple flesh, is the carotenoid in nature with the highest antioxidant capacity, in special for its cis–isomers. Tomato skin is a low cost by-product with very high lycopene content (7.23 g kg^{-1}) and high potential to be incorporated as an antioxidant agent in antibrowning dipping treatments. High lycopene extraction from tomato skin was achieved with a thermal treatment (75 ºC, 1 h) favouring trans–to–cis lycopene isomerization with TiO_2 nanoparticles. Lycopene extracts were highly encapsulated (encapsulation efficiency: 92.2 %) with the complex coacervation method and the obtained microspheres were then incorporated in dipping treatments (0.5 (L0.5), 1 (L1) or 2 g L^{-1} (L2)) during fresh–cut

apples processing, compared with an ascorbic acid dipping (AA; 10 g L^{-1}). Quality changes (colour, microbial, physicochemical and bioactive compounds) were studied up to 9 d at 5 ºC. The L2 dipping controlled better the browning during storage, showing the lowest browning index among treatments (BI=43.8) after 9 d. Furthermore, L2 dipping did not affect the physicochemical quality of samples, while maintaining a good microbial quality. Incorporation of lycopene microspheres also improved the bioactive quality of samples, still showing total cis−lycopene isomer content of ≈ 20 mg kg^{-1} after 9 d. Furthermore, chlorogenic acid, the predominant phenolic acid, content was enhanced by 56 % in L2 samples after 6–9 d. In conclusion, a dipping treatment of fresh–cut apples including 2 g L^{-1} lycopene microspheres reduced browning, while quality was maintained and some bioactive compounds even enhanced after 9 d at 5 ºC.

Keywords: lycopene isomerization; nanoencapsulation; browning; TiO_2 nanoparticles; antioxidant; phenolic compounds.

1. INTRODUCTION

Fresh–cut apples still remain a challenge for food technologists, mainly due to the enzymatic browning, which highly influences the consumer decision among the rest of sensory parameters (Toivonen and Brummell, 2008). During processing, membranes inside cells of apples are disrupted mixing the polyphenol oxidase (PPO) with phenolic substrates. As a result, PPO oxidases phenolic compounds through two reactions: monophenols>diphenols hydroxylation and diphenols>quinones oxidation. The formed coloured quinones follow further reactions leading to melanin, which is the pigment responsible of brown and black colour of fresh–cut apples with high browning incidence (Cortellino et al., 2015; Toivonen and Brummell, 2008). Particularly, susceptibility to

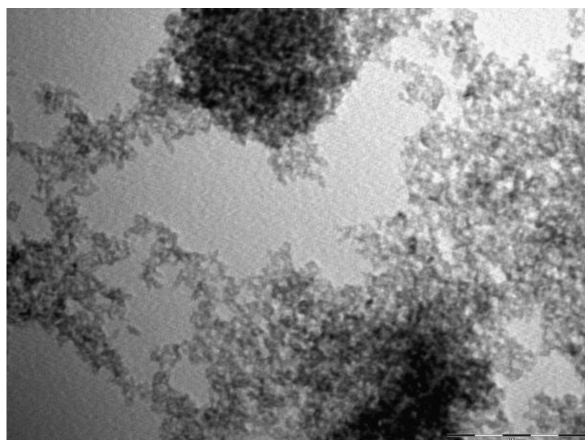
51 browning is highly dependent of the apple variety, showing 'Braeburn' and 'Fuji'
52 varieties the highest browning rates (Falguera et al., 2013; Luo and Barbosa-Cánovas,
53 1997).

54 Modified atmosphere packaging (MAP) of fresh–cut apples under O_2/CO_2 partial
55 pressures of 1−5 / 7−20 kPa preserves firmness while limits the ethylene production
56 (Cortellino et al., 2015; Raybaudi-Massilia et al., 2007; Salvia-Trujillo et al., 2015).
57 Furthermore, low O_2/high CO_2 partial pressures achieved during MAP limit microbial
58 growth extending the product shelf life. Nevertheless, the recommended O_2 and CO_2
59 concentrations are not able to control enzymatic browning of fresh−cut apples if MAP is
60 not combined with antibrowning agents (Cortellino et al., 2015; Rojas-Graü et al., 2009).
61 The main antibrowning agents already studied in fresh–cut apples have been: ascorbic
62 acid, thiol–containing compounds (N–acetylcysteine and reduced glutathione),
63 carboxylic acids (citric, oxalic, etc.), phenolic acids (e.g. kojic acid), resorcinols (4–
64 hexylresorcinol), and their combinations, among others (Oms-Oliu et al., 2010).
65 Encapsulation of apple phenolic compounds with cyclodextrins has also been proposed
66 by our group to avoid the substrate–PPO contact highly reducing the product browning
67 in apple juice (Martínez-Hernández et al., 2019). Ascorbic acid is the most frequent
68 antibrowning agent used for fresh–cut apples due to its low cost, safety and effectiveness
69 (EFSA, 2015; FDA, 2018). Accordingly, ascorbic acid has been incorporated into
70 antibrowning dipping treatments (usually at 5–10 g L^{-1}) for fresh–cut fruit for more than
71 two decades (Baldwin et al., 1996; Oms-Oliu et al., 2010). The antibrowning effect of
72 ascorbic acid is due to its ability to reduce the o–quinones back to their phenolic
73 precursors (Hsu et al., 1988; Toivonen and Brummell, 2008). Nevertheless, the
74 antibrowning effect of ascorbic acid is limited since once ascorbic acid has been
75 completely oxidized to dehydroascorbic acid, coloured quinones are formed again leading

76 to browning (Rojas-Graü et al., 2006). Therefore, natural antibrowning treatments
77 alternative to ascorbic acid are needed.
78 Tomato is an excellent source of lycopene: the carotenoid with the highest antioxidant
79 capacity in nature (Böhm et al., 2002; Martínez-Hernández et al., 2016). In particular,
80 tomato skin has ≈14–fold higher lycopene content than internal tissues (Moco et al.,
81 2007). Nevertheless, lycopene is highly degraded under O_2 and high temperatures,
82 although encapsulation may reduce these losses while allowing a controlled lycopene
83 release during time. In this sense, lycopene was successfully encapsulated using the
84 complex coacervation method showing the obtained microspheres a lycopene degradation
85 lower than 5 % after 10 d at 10 ºC (Rocha-Selmi et al., 2013).
86 Attending to the carotenoid structure, cis–lycopene isomers have higher antioxidant
87 capacity than all–trans lycopene (Böhm et al., 2002). Furthermore, cis–lycopene isomers
88 have higher bioavailability in the human intestine than all–tans lycopene (Boileau et al.,
89 1999). Trans–to–cis isomerization is then preferred during processing of tomato
90 (Martínez-Hernández et al., 2016). A trans–to–cis lycopene isomerization of ≈ 83 % was
91 achieved using TiO_2 nanoparticles, being these nanoparticles easily removed with
92 filtration or centrifugation avoiding contamination or harmful to the food (Sun et al.,
93 2016).
94 Tomato varieties with higher lycopene contents have shown lower PPO activity (Spagna
95 et al., 2005). Furthermore, lycopene is an antioxidant agent that reconstitutes the
96 polyphenols oxidized by the action of PPO leading to lower colour changes as observed
97 Spagna et al. (2005) in tomatoes. Therefore, the use of lycopene as an antibrowning agent
98 in fresh–cut apples is of high interest.
99 Reduction of fruit and vegetable waste has gained a high, a needed, interest in order to
100 combat global hunger and improve food security (Porat et al., 2018). In this sense,

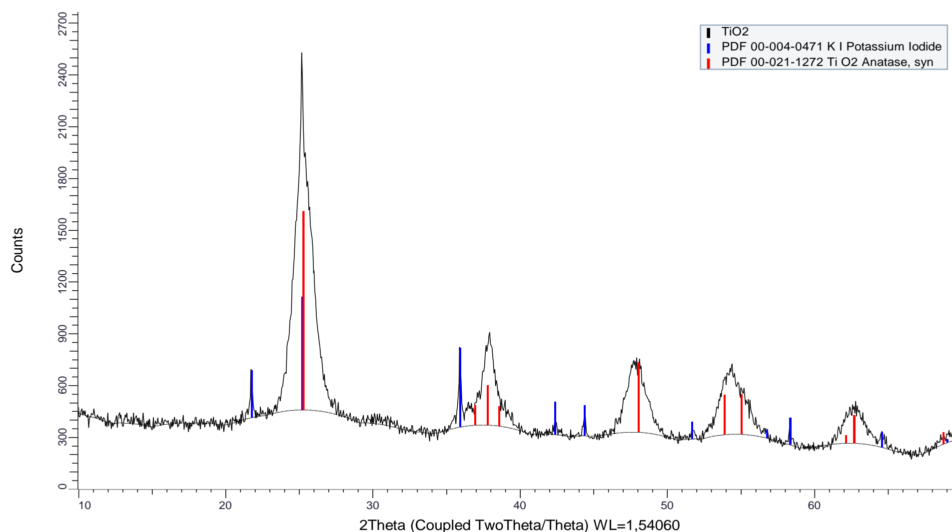
101 revalorization of tomato by-products from the processing industry is of high interest due
102 to the low cost of this rich lycopene source while minimising the environmental impact.
103 The use of tomato by-products as a lycopene source to maintain quality and improve
104 healthiness of food has been already studied in meat and bakery products (Rocha-Selmi
105 et al., 2013; Viuda-Martos et al., 2014). Nevertheless, to the best of our knowledge, the
106 incorporation of lycopene in fresh–cut products to preserve their quality, together with
107 the enhancement of the product healthiness, has not been studied yet.
108 The aim of the present study was to investigate the effects of dipping solutions including
109 cis–lycopene microspheres, obtained from tomato by-products (skin), on the
110 physicochemical, microbial and bioactive quality of fresh–cut apples during storage at 5
111 ºC up to 9 d. This innovative antibrowning dipping was compared to a conventional
112 dipping with ascorbic acid.
113
114 **2. MATERIAL AND METHODS**
115 **2.1. Plant material and preparation of TiO_2 nanoparticles**
116 Apples (*Malus domestica* cv. Braeburn) were purchased from a local supermarket
117 (Cartagena, Spain) in November 2017. Fruit was originally harvested in orchards from
118 the Bozen–Etsch Valley area (Terlan, Italy) and packaged in macroperforated plastic bags
119 (7 kg of apples per bag) in the installations of Fruit Growers' Cooperative (VOG, Terlan,
120 Italy). Fruit was classified by the company as category I (80–85 mm diameter).
121 Plum tomatoes (*Solanum lycopersicum* L. var. lycopersicum cv. canilesTM) were grown
122 in the Southeast of Spain (Agrícola Gaobe S.L., Almería) under greenhouse conditions
123 according to integrated pest management cultural practices. Tomatoes were harvested on
124 November 2017 at mature physiological stage with a size of 42–72 mm and red colour.
125 Tomatoes were transported ≈190 km to the Pilot Plant of the Institute of Plant

126 Biotechnology. Tomatoes and apples were stored at 5 ºC and 90–95 % relative humidity
127 (RH) until the next day when they were processed.
128 TiO_2 nanoparticles were prepared with the hydrothermal method described by Sun et al.
129 (2016). Briefly, 6 mL of solution A (5 mL of tetrabutyl titanate reagent (97 % purity) + 1
130 mL of acetic acid) was added dropwise into 60 mL of aqueous solution B (4.05 g L^{-1} of
131 KI and 0.17 g L^{-1} of polyvinylpyrrolidone (PVP)) with vigorous stirring, which was
132 continued for 4 h at room temperature. Hydrothermal reaction of the latter mixture was
133 conducted for 24 h at 100 ºC using a Teflon−lined autoclave (Shilpent Auto, Pekin,
134 China). The obtained precipitate was dried in a vacuum oven (80 ºC, 12 h), calcined (185
135 ºC, 2 h), and finally ground to a fine powder.
136 Characterization of TiO_2 nanoparticles was made as follows. The structure of TiO_2
137 nanoparticles was observed by transmission electron microscopy (TEM; Philips Tecnai
138 12 microscope (Amsterdam, Netherlands)) and X−ray diffraction (XRD; Anton–Paar
139 SAXSess diffractometer (Anton Paar GmbH, Graz, Austria) with CuKα radiation). TEM
140 images (Supplementary material 1) showed a morphology similar to Sun et al. (2016)
141 with a nanoparticle size of 20−30 nm. XRD patterns (Supplementary material 2)
142 displayed the typical anatase titania structure of TiO_2 nanoparticles with characteristic
143 peaks at 2θ value of 25º, 38º, 48º, 54.5º y 63º, as previously reported (Ma et al., 2011;
144 Sun et al., 2016).

145 

146 **Supplementary material 1.** TEM images of TiO_2 nanoparticles.

147



148

149 **Supplementary material 2.** XRD patterns of TiO_2 nanoparticles.

150

151 **2.2. Lycopene extraction from tomato skin**

152 Tomatoes were sanitized (NaClO, 100 mg L^{-1}; 5 ºC; pH 6.5; 1 min), rinsed (tap water; 5
153 ºC; 1 min) and then drained in a perforated basket for 1 min. Sanitised tomatoes were
154 peeled with a manual tomato peeler (Tescoma, Alicante, Spain), and then frozen with
155 liquid nitrogen, and stored at -80 ºC until lycopene extraction. Tomato peeling in the
156 tomato product industry is different (mainly chemical peeling) obtaining a final tomato
157 paste comprising all tomato waste (including seeds). Nevertheless, we decided to peel
158 tomatoes manually in order to avoid experimental interferences with residual chemicals
159 (NaOH, additives, etc.) used during industrial peeling of tomatoes.

160 Different lycopene extraction treatments from tomato skins were defined according to
161 literature (Ho et al., 2015; Kumcuoglu et al., 2014; Sun et al., 2016). Ethyl acetate (100
162 %) was used as extraction solvent according to Ho et al. (2015) due to the high lycopene
163 extraction yields with this solvent. The tomato skin:ethyl acetate ratio during extraction

164 treatments was 1:4 (*w:v*) according to Ho et al. (2015). The 4 studied extraction treatments
165 were:
166 • Thermal extraction (75 ºC, according to Celli et al. (2016) and Sun et al. (2016))
167 for 1 or 2 h.
168 • Ultrasounds extraction (30 min, according to Kumcuoglu et al. (2014)) at ≈0 ºC
169 (ice−water) or room temperature.
170 Trans−to−cis isomerization during extraction treatments with TiO_2 nanoparticles was also
171 studied including 50 mg of TiO_2 nanoparticles per 250 g of tomato skin, as previously
172 optimized by Sun et al. (2016). A control extraction treatment without TiO_2 nanoparticles
173 was also conducted for each of the 4 extraction methods. After extraction treatments, the
174 lycopene content from the ethyl acetate extracts was analysed to select the optimum
175 extraction method.
176 The obtained lycopene extract was centrifuged (5,000 × *g*, 15 ºC, 10 min) and then dried
177 with a rotary evaporator (70 ºC). The dried extracts from 6 extraction batches (total
178 tomato skin = 1.5 kg) were resuspended in 5 mL of sunflower oil (Koipe, Madrid, Spain)
179 (Celli et al., 2016) obtaining a cis−lycopene−rich oil. The cis−lycopene−rich oil was
180 stored in 10−mL amber vials, flushed with N_2, until the encapsulation procedure.
181
182 **2.3. Preparation of lycopene microspheres and characterization**
183 A gelatine–pectin complex coacervation procedure (Rocha-Selmi et al., 2013; Silva et al.,
184 2012) was used for the encapsulation of the obtained cis−lycopene−rich oil. Briefly, the
185 cis−lycopene−rich oil (5 mL) was dropwise added to 50 mL of preheated (50 ºC) gelatine
186 solution (25 g L^{-1}), and then vigorously stirred for 3 min at 50 ºC. Subsequently, the latter
187 emulsion was mixed with 50 mL of preheated (50 ºC) gum Arabic solution (25 g L^{-1})
188 under vigorously stirring at 50 ºC. The pH was then adjusted to 4.0 with 0.5 M HCl. The

189 temperature was gradually reduced to: (1) 25 ºC, (2) 10 ºC (with an ice bath) and then (3)
190 to 3 ºC (24 h in cold chamber at 3 ºC) to complete particle precipitation. Finally,
191 microspheres of cis−lycopene−rich oil (hereinafter 'lycopene microspheres') were
192 filtered (25 μm) and freeze−dried.

193 The morphology of lycopene microspheres was observed using an optical microscopy
194 (Leica CLS 150 XD, Madrid, Spain) at 100× magnification.

195 The encapsulation efficiency (EE) was also calculated (Eq. 1) as the amount of lycopene
196 present in the microcapsules compared with the initial lycopene quantity used to produce
197 them. Prior to EE determination, the microcapsules were ruptured according to Silva et
198 al. (2012). Briefly, 10 mg of microcapsules were dispersed in 10 mL of NaCl (11.69 g
199 L^{-1}), vortex and then allowed to rest for 30 min. Subsequently, 10 mL of petroleum ether
200 and 10 mL of ethanol were slowly added, vortex and then allowed to rest for another 5
201 min. The obtained solution was centrifuged and filtered. The lycopene content of the
202 solution with the disrupted microspheres was analysed (see lycopene analysis section).
203 Each experiment was carried out in triplicate.

204 $$EE\ (\%) = \frac{M_a}{M_{th}} \times 100 \qquad (1)$$

205 Where M_a is the actual amount of lycopene entrapped in the microspheres and M_{th} is the
206 theoretical amount of lycopene entrapped in the microspheres.

207 The encapsulation yield (EY) was calculated as described in Eq. (2).

208 $$EY\ (\%) = \frac{M_{sa}}{M_{sb}} \times 100 \qquad (2)$$

209 where M_{sa} is the total mass of microcapsules obtained after encapsulation and drying, and
210 M_{sb} is the total mass of solids before encapsulation.

211

212 **2.4. Fresh-cut processing of apples and lycopene microspheres incorporation**

213 Fresh-cut apples processing was conducted in a disinfected cold room (8 ºC). Apples were
214 sanitized (NaOCl, 100 mg L^{-1}; 5 ºC; pH 6.5; 1 min), rinsed (tap water; 5 ºC; 1 min) and
215 then drained in a perforated basket for 1 min. Sanitised apples were cut into wedges (8
216 wedges per apple) with a manual apple wedge–cuter (Tescoma, Alicante, Spain). Apple
217 wedges were dipped in the following solutions, which represented the treatments of the
218 experiment:

219 - **L0.5**: dipping solution containing 0.5 g of lycopene microspheres per L.
220 - **L1**: dipping solution containing 1 g of lycopene microspheres per L.
221 - **L2**: dipping solution containing 2 g of lycopene microspheres per L.
222 - **AA**: a control antibrowning dipping with ascorbic acid at 10 g L^{-1}. The ascorbic
223 acid concentration was selected according to Soliva-Fortuny et al. (2001).
224 - **CTRL**: as control, dipping with water was conducted.

225

226 The highest lycopene microsphere concentration (2 g L^{-1}) was selected (during
227 preliminary dipping treatments) as the maximum concentration that did not confer a
228 red/orange colour (from lycopene) to apple flesh, which would lead to a consumer
229 rejection of the product. All dipping solutions were prepared with stirring for 5 min at
230 room temperature. Samples were dipped for 8 min in the treatment solutions and then
231 allowed to rinse on a towel paper for 5 min as previously described (Ortiz-Duarte et al.,
232 2019). A control dipping was made with water.
233 Treated samples (8 wedges; ≈120 g) were placed in rectangular trays (120×78×45 mm;
234 370 mL). Sanitized (70 % ethanol) plastic nets (1 mm of height) were previously placed
235 on the bottom of each tray to allow for the draining of excess dipping treatments and to
236 minimize related quality losses and microbial growth. Then, trays were thermally sealed
237 on the top with a bioriented polypropylene (BOPP) film of 40 µm thickness (Plásticos del

238 Segura S.L., Murcia, Spain). The permeability of the BOPP film at was 800 cm^3 m^{-2} d^{-1}

239 atm^{-1} (O_2) (23 ºC, 0 % RH; data provided by the supplier). Samples were stored at 5 ºC

240 (90–95 % RH) in darkness with sampling times of 0, 3, 6 and 9 d. Three replicates per

241 treatment and sampling day were prepared.

242

243 **2.5. Gas analysis within modified atmosphere packages**

244 O_2 and CO_2 partial pressures of MAP were monitored during storage at 5 ºC. The

245 headspace gas samples (1 mL) were withdrawn from the packages and were analysed in

246 a gas chromatography (GC; PerkinElmer Precisely Clarus 500, Massachusetts, USA).

247 The GC conditions for O_2 and CO_2 determinations have been described Álvarez-

248 Hernández et al. (2019). Two readings per tray were analysed on each sampling day prior

249 to the rest of analyses.

250

251 **2.6. Colour and physicochemical analyses**

252 The colour was determined using a colorimeter (Chroma Meter CR–400, Konica Minolta;

253 Tokyo, Kanto, Japan) set at Illuminant D65. The colorimeter was calibrated with a white

254 reference plate 2º observer with 8 mm viewing aperture. Measurements were obtained

255 using the standard tristimulus CIE $L^*a^*b^*$. Four colour readings (2 readings for each side

256 of apple wedge) were taken and automatically averaged by the device. Five apple wedges

257 were measured per each tray. Browning index (BI) was calculated from the CIE $L^*a^*b^*$

258 parameters according to Eq. (3) as previously described (Palou et al., 1999).

259 $$BI = \frac{100 \times \left[\left[\frac{[a^2 + (1.75 \times L)]}{[(5.645 \times L) + a^2 - (3.012 \times b)]}\right] - 0.31\right]}{0.172} \quad (3)$$

260

261 Juice from apple wedges was obtained by grinding 5 wedges (per tray) with a blender
262 (MX2050 blender, Braun, Germany). Soluble solid content (SSC) was determined with a
263 digital hand–held refractometer (Atago N1; Tokyo, Kanto, Japan) at 20 ºC and was
264 expressed as %. The pH was measured with a pH–meter (Basic20, Crison; Alella,
265 Cataluña, Spain). The titratable acidity (TA) of diluted juice (5 mL plus 45 mL of distilled
266 water) was determined by titration (T50, Metter Toledo; Milan, Italy) with 0.1 M NaOH
267 to pH 8.1 and was expressed as malic acid in g L^{-1}. Each of the three replicates were
268 analysed in duplicate.
269

270 **2.7. Microbial analyses**

271 Standard enumeration methods were used to determine mesophilic, psychrophilic,
272 enterobacteria and yeast and mould growth (Martínez-Hernández et al., 2013; Tomás-
273 Callejas et al., 2012). All used microbial media was acquired from Scharlau Chemie
274 (Barcelona, Spain). The following media and incubation conditions were used: Plate
275 Count Modified Agar for mesophilic and psychotropic aerobic bacteria with incubations
276 of 30 ºC/48 h and 5 ºC/7 d, respectively; Violet Red Bile Dextrose Agar for enterobacteria
277 with an incubation of 37 ºC/48 h; and Rose Bengal Agar for yeasts and moulds (Y+M)
278 with an incubation of 22 ºC/7 d. All microbial counts were reported as log colony forming
279 units per gram of product (log CFU g^{-1}). Each of the three replicates was analysed in
280 duplicate. The presence of *Salmonella* spp., *Listeria monocytogenes* and generic
281 *Escherichia coli* was monitored according to the European legislation for this kind of
282 plant products (EC, 2007).
283

284 **2.8. Lycopene content**

285 Lycopene content was analysed according to Gupta et al. (2015). Briefly, 1.5 mL of
286 chloroform:dichloromethane (2:1, *v:v*) was added to 150 mg of freeze–dried sample, and
287 then it was mixed for 20 min at 4 ºC with an orbital shaker at 1,000 rpm. Then, 0.5 mL of
288 NaCl at 58.5 g L^{-1} was added, mixed by inversion and then centrifuged (5,000×g, 10 min,
289 4 ºC). The organic phase was collected and the aqueous phase was re–extracted with 0.75
290 mL of chloroform:dichloromethane (2:1, v/v) twice, and then the three organic phases
291 were pooled and dried under N_2. Finally, dried extracts were re–dissolved in 1 mL of
292 methanol/tert–butyl methyl ether (MTBE) (25:75, *v:v*) and filtered with a
293 polytetrafluoroethylene (PTFE) syringe filter of 0.22 μm.
294 An ultra–high–performance liquid chromatography (UHPLC) instrument (Shimadzu,
295 Kyoto, Japan) equipped with a DGU–20A degasser, LC–170 30AD quaternary pump,
296 SIL–30AC autosampler, CTO–10AS column heater and SPDM–20A diode array detector
297 (DAD) was used. Chromatographic separation was carried out using a C30 column (250
298 × 4.6 mm; 3 μm) (YMC Co., Kyoto, Japan), coupled to a 20×4.6 mm C30 guard column,
299 at 20 ºC. The mobile phases were (A) metanol:water (98:2, *v:v*), (B) methanol:water
300 (95:5, *v:v*) and (C) MTBE. The gradient elution started with 80 % A, 20 % C at 0 min,
301 followed by linear gradient to 60 % A, 40 % C to 2.00 min at a flow rate of 1.4 mL min^{-}
302 ^{1}, at 2.01 min flow rate was changed to 1.00 mL min^{-1} with gradient changing to 60 % B,
303 40 % C followed by a linear gradient to 0 % B, 100 % C by 12 min and return to initial
304 conditions by 13.00 min. UV–visible detection was performed at 476 nm. Lycopene
305 isomers were identified according to Gupta et al. (2015) based on: (1) main absorption
306 peaks, (2) Q–ratio (height ratio of the cis–peak to the main absorption peak) and (3) %
307 III/II ratio (ratio of peak heights from the trough between peak II and III). All–trans
308 lycopene was quantified with an authentic standard (Carotenature, Münsingen,
309 Switzerland) and expressed as mg kg^{-1} (dry weight basis). Lycopene cis–isomers were

310 also quantified with the all–trans lycopene standard because of similarity in extinction
311 coefficient (Gupta et al., 2015; Lin and Chen, 2003).
312
313 **2.9. Phenolic compounds**
314 A unique extract for phenolic compounds and total antioxidant capacity (TAC) was done.
315 Briefly, 5 g of freeze–dried sample was mixed with 10 mL of acidified (10 mL L^{-1} of
316 formic acid) 50 % methanol acidified and homogenised (UltraTurrax T25 basic, IKA,
317 Germany) for 10 s. Samples were then centrifuged (15,000 × *g*, 15 min, 4 ºC) and the
318 supernatants were used as the phenolic compounds/TAC extracts. Extracts for
319 determination of individual phenolic compounds by UHPLC were further filtered (0.22–
320 µm PTFE syringe filter).
321
322 2.9.1. Total phenolic content (TPC)
323 The Folin–Ciocalteu reagent method was used to analyse the TPC as previously described
324 (Martínez–Hernández et al., 2011). Briefly, a 22 µL aliquot of the diluted (50 %) TPC
325 extract was placed on a flat–bottom PS 96–well plate (Greiner Bio–One, Frickenhausen,
326 Germany) and 29 µL of 1 N Folin–Ciocalteu reagent (Sigma, St Louis, MO, USA) was
327 added. The latter mixture was incubated for 3 min at room temperature in darkness. After
328 incubation, 192 µL of a mix solution (4.03 g L^{-1} Na_2CO_3 and 20.0 g L^{-1} NaOH) was
329 added and the reaction was carried out for 1 h at room temperature in darkness. Then,
330 absorbance was measured at 750 nm using a Multiscan plate reader (Tecan Infininte
331 M200, Männedorf, Switzerland). The TPC was expressed as gallic acid equivalents in g
332 kg^{-1} (dry weight basis). Each of the three replicates was analysed in triplicate.
333
334 2.9.2. Individual phenolic content

335 The phenolic extracts were analysed using the UHPLC instrument (Shimadzu, Kyoto, 336 Japan) with the method of Février et al. (2017). Chromatographic analyses were carried 337 out using a Gemini C18 column (250 mm × 4.6 mm, 2.6 mm particle size; Phenomenex, 338 Macclesfield, UK) at 30 ºC. The mobile phases were water (A) and acetonitrile (B), both 339 acidified with formic acid (1 mL L^{-1}). The elution gradient started with 3% B; 0–3 min: 340 7% B linear; 3–21 min: 13% B linear; 21–27 min: 13% B linear; 27–41 min: 20% B 341 linear; 41–51 min: 45% B linear; 51–53 min: 90% B linear; 53–56 min: 90% linear; and 342 then the column was washed and reconditioned. The flow rate was 1 mL min^{-1}. UV– 343 visible detection was performed at 320 and 280 nm for chlorogenic acid and flavonoids, 344 respectively. Phenolic compounds were identified and quantified, in mg kg^{-1} (dry weight 345 basis), with commercial standards (Sigma–Aldrich, St. Louis MO, USA). Each of the 346 three replicates was analysed in duplicate.

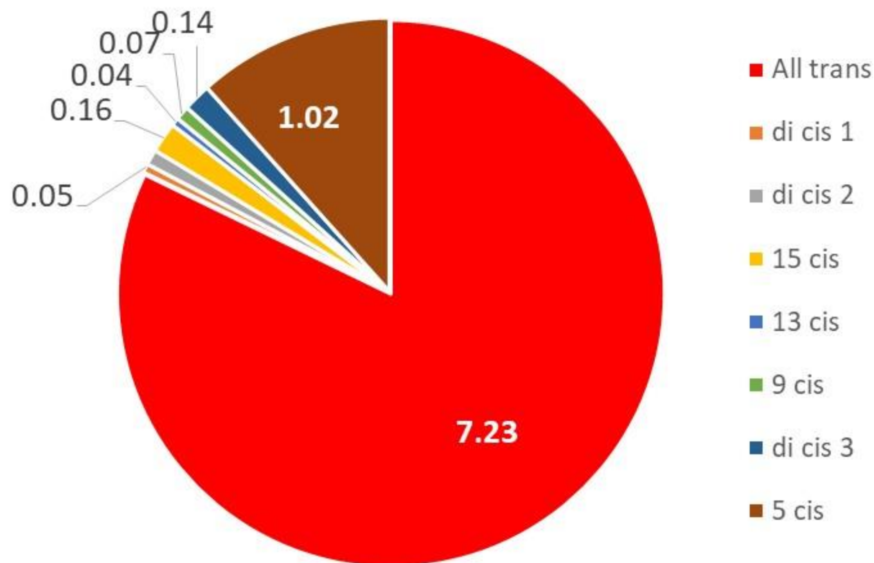
347

348 **2.10. Total antioxidant capacity**

349 TAC was determined as described by Klug et al. (2018) by three different methods: free 350 radical scavenging capacity with 2,2–diphenyl–1–picrylhydrazil (DPPH) (Brand-351 Williams et al., 1995), ferric–reducing antioxidant power (FRAP) (Benzie and Strain, 352 1999), and 2,20–azino–bis (3–ethylbenzothiazoline–6–sulphonic acid) (ABTS) (Cano et 353 al., 1998). DPPH method was conducted by measuring the decrease in absorbance at 515 354 nm after 30 min. A volume (194 µL) of DPPH solution (≈ 0.7 mM and adjusted to 355 Abs_{515nm} = 1.1 ± 0.02) was added the diluted (30:70 sample extract: extract solvent, *v:v*) 356 TAC extract (21 µL) and allowed to react for 30 min. FRAP method was conducted by 357 measuring the increase in absorbance at 593 nm for 45 min. The freshly made–up FRAP 358 solution (prepared in 10:1:1 v:v:v proportion of sodium acetate buffer, pH 3.6; 10–mM 359 TPTZ solution in 40–mM HCl; and 20–mM $FeCl_3$, respectively, and preincubated at 37

360 ºC for 2 h) was added (198 μL) to the diluted (70:30, *v:v*) TAC extract (6 μL) and allowed
361 to react for 30 min. ABTS method was conducted by measuring absorbance increase at
362 734 nm for 60 min. A volume (280 μL) of ABTS solution (14–mM $ABTS^+$ and 4.9–mM
363 $K_2S_2O_8$ by 1:1 (v:v)) was added to the diluted (30:70, *v:v*) TAC extract (6 μL) and allowed
364 to react for 45 min. All TAC reactions were conducted at room temperature in darkness
365 and absorbances were measured using the same microplate reader for TPC. TAC data
366 were expressed as Trolox equivalents in mg kg^{-1} fw. Each of the three replicates was
367 analysed in triplicate.
368
369 **2.11. Statistical analyses**
370 The experiment had a two−factor (treatment×storage time) design subjected to analysis
371 of variance (ANOVA) using the SPSS software (v.19 IBM, New York, USA). Statistical
372 significance was assessed at p=0.05, and the Tukey's multiple range test was used to
373 separate the means.
374
375 **3. RESULTS AND DISCUSSION**
376 **3.1. Lycopene extraction from tomato by-products and trans–to–cis isomerization**
377 **with TiO_2 nanoparticles**
378 Tomato skin showed an all−trans lycopene content of 7.23 g kg^{-1} (Figure 1). The all−trans
379 lycopene content in tomato skin is ≈14−fold higher than in the remaining internal tomato
380 tissues (at red ripening stage) according to data from Moco et al. (2007). In that sense,
381 tomato skin is widely considered as a lycopene−rich by-product. Attending to
382 cis−lycopene isomers, tomato skin showed a total content of cis−lycopene isomers of 1.57
383 g kg^{-1}, which was distributed as follows: 65.2 % 5−cis, 10.1 % 15−cis, 9.2 % di cis 3, 5.1
384 % di cis 2, 4.7 % 9−cis, 3.0 % di cis 1 and 2.7 % 13−cis (Figure 1). Gupta et al. (2015)
385 also found 5−cis as the main cis isomer in red tomato.

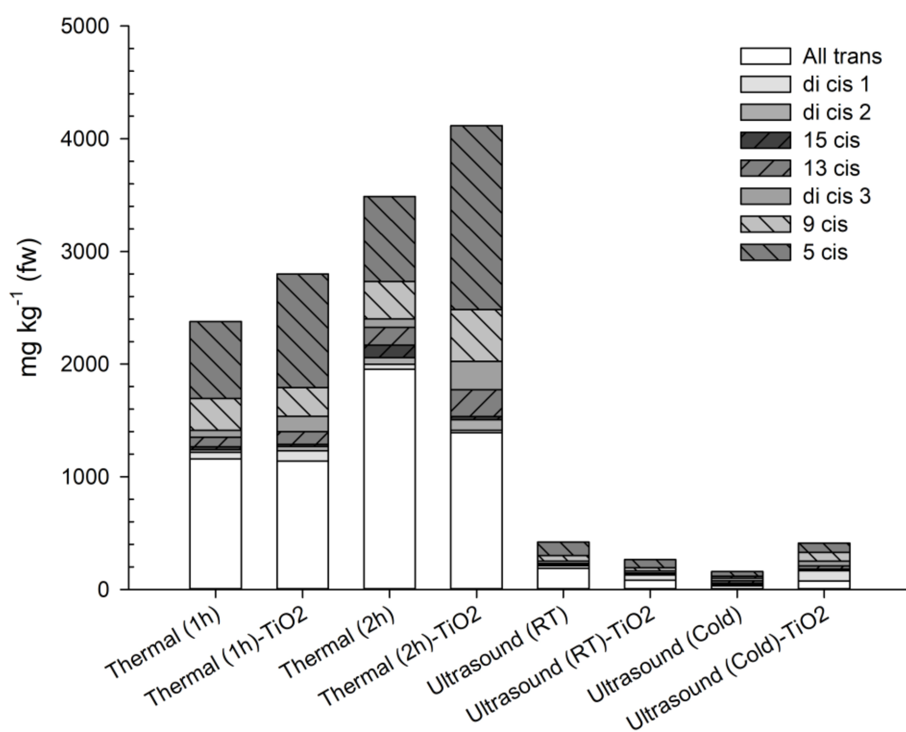
386



387
388 **Figure 1.** Contents of lycopene isomers (g kg^{-1}) in tomato skin.

389

390 Thermal treatments achieved a higher lycopene extraction from tomato skin compared
391 with ultrasound treatments (Figure 2). Particularly, the ultrasound treatment with
392 ice−water bath extracted 5−fold lower all−trans lycopene content regarding the
393 ultrasound treatment at room temperature. Nevertheless, Kumcuoglu et al. (2014)
394 reported a higher lycopene extraction using an ultrasound treatment (24 Hz, 90 W, 30
395 min, 5 ºC; 2:1:1 (*v:v:v*) hexane: methanol:acetone) compared to a thermal treatment (60
396 ºC, 40 min; 2:1:1 (*v:v:v*) hexane: methanol:acetone). The latter finding from Kumcuoglu
397 et al. (2014) may be explained since tomato by-products were already vacuum−dried prior
398 to extraction treatments. Then, lycopene from such disrupted (due to vacuum−drying)
399 plant cells was more available and subsequently more susceptible to thermal degradation
400 leading to the observed higher lycopene yields with the ultrasound extraction.
401 Nevertheless, drying, and specially vacuum−drying, is a preparative technique that
402 increases the industrial costs (electricity, vacuum system, etc.) and delays the extraction
403 procedure.



404

405 **Figure 2.** Contents of lycopene isomers (g kg^{-1}) extracted from tomato skin using
406 different extraction treatments.

407

408 The TiO_2 nanoparticles improved the trans−to−cis lycopene isomerization by 36 and 78
409 % during the 1 h− and 2 h−thermal extractions, respectively (Figure 2). Similarly, Sun et
410 al. (2016) increased the trans−to−cis lycopene isomerization during a thermal treatment
411 (75 ºC, 2 h; ethyl acetate) from 38 to 83 % using TiO_2 nanoparticles. The 5−cis isomer
412 showed the highest content among all cis isomers after the TiO_2−extraction (2 h) followed
413 by the 9−cis isomer with 59 and 17 %, respectively (Figure 2). In that sense, thermal (75
414 ºC) extraction of lycopene from tomato skin for 2 h using TiO_2 nanoparticles was selected
415 as the method with higher lycopene yields and trans−to−cis isomerization rate.
416 After resuspension of the dried (rotary evaporator) lycopene−rich extract in the oily phase
417 (3 mL of sunflower oil), the obtained lycopene−rich oil showed a total lycopene content
418 of 57.2 mg mL^{-1} (data not shown).

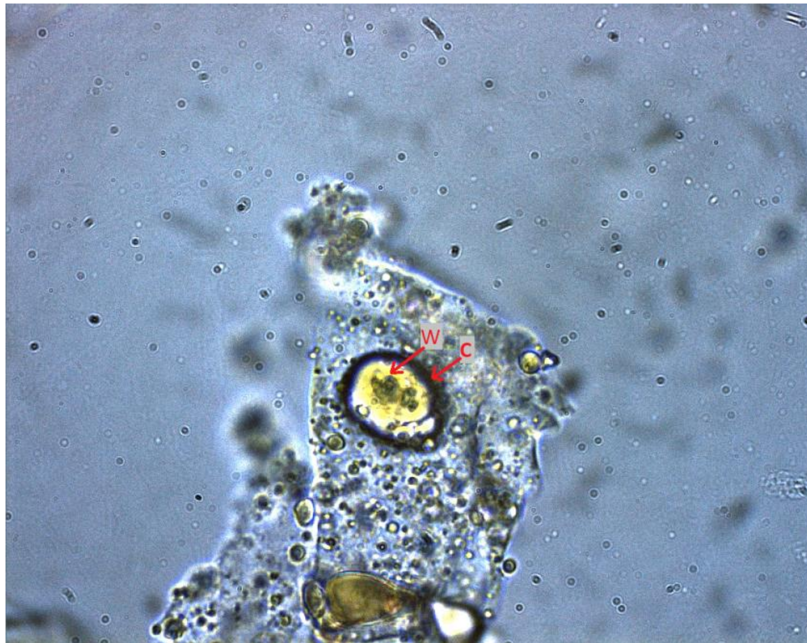
419

420 **3.2. Characterization of lycopene microspheres**

421 The core:wall material ratio is the most important parameter, followed by the ratio
422 between wall materials (gelatine:gum Arabic ratio), to obtain microspheres with a high
423 EE and a spherical structure that facilitates the flow and controls the release of the core
424 material (Nori et al., 2011; Wu et al., 2013). The obtained microspheres showed EE and
425 EY of 92.2 and 60.0 %, respectively, which are similar to previous data for lycopene
426 microspheres obtained using the same coacervation method (Silva et al., 2012; Wu et al.,
427 2015). Lycopene microspheres, with similar EE to our microspheres, showed a low
428 lycopene degradation of 23.3 % after 73 d at 10 ºC while lycopene in its free form was
429 degraded by 38.8 % after such storage period (Rocha-Selmi et al., 2013). Furthermore,
430 microspheres obtained with this coacervation procedure have revealed a controlled
431 release of the core material with a minimum burst effect (Wu et al., 2013).

432 The microscopic analysis of samples showed a spherical morphology with defined core
433 and wall structures (Figure 3). Similar structures with comparable sizes (10−90 µm) have
434 been reported in lycopene and propolis microspheres using the same coacervation method
435 (Nori et al., 2011; Silva et al., 2012).

436

437 

438 **Figure 3.** Image obtained by optical microscopy of lycopene microspheres. W: wall
439 material; C: core material.

440

441 In conclusion, the used core:wall material (1:1) and gelatine:gum arabic (1:1) ratios
442 allowed to obtain lycopene microspheres with a high EE and an excellent microscopic
443 morphology, which may permit a controlled lycopene liberation as previously
444 characterized (Silva et al., 2012).

445

446 **3.3. Gas analysis within modified atmosphere packages**

447 Packages of fresh−cut apples under MAP reached equilibrium O_2/CO_2 partial pressures
448 of 8.0−10.2/8.5−10.2 after 6 d. No significant ($p>0.05$) treatment effect was observed
449 during storage (data not shown). The apple firmness was preserved using MAP O_2/CO_2
450 partial pressures of 1−5/7−20 kPa while the ethylene production was limited (Cortellino
451 et al., 2015; Raybaudi-Massilia et al., 2007; Salvia-Trujillo et al., 2015). Accordingly, the
452 reached gases partial pressures after 6−9 d may ensure the quality of fresh−cut apples
453 related to firmness while avoiding the deleterious effects of ethylene in this fruit, which

454 has well−known high ethylene production rates. Nevertheless, the achieved O_2 and CO_2
455 partial pressures during apple MAP are not able to control enzymatic browning if MAP
456 is not combined with anti−browning dipping treatments (e.g. ascorbic acid) as previously
457 reviewed (Cortellino et al., 2015; Rojas-Graü et al., 2009).
458
459 **3.4. Colour**
460 Enzymatic browning of fresh−cut apples after processing and during storage is
461 characterized by an increment of a^* and b^* parameters, while L^* decreases (Pérez–Gago
462 et al., 1999). Nevertheless, BI is recommended as a good indicator of enzymatic browning
463 in fresh−cut apples since it effectively combines and reflects L^*, a^* and b* changes
464 related to enzymatic browning (Pérez–Gago et al., 1999).
465 Enzymatic browning in fresh−cut apples is known to occur very fast, showing CTRL
466 samples a BI of ≈40 on processing day (Table 1). However, lycopene microspheres
467 reduced ($p<0.05$) the burst browning of fresh−cut apples on day 0 to BI of 35 and 30 in
468 L0.5, L1 and L2 samples, respectively. Such browning reduction was similar to that
469 achieved with the ascorbic acid dipping on day 0 (BI=33) (Table 1). Nevertheless, the
470 initial antibrowning effect of AA was limited being not observed after 3 d, with similar
471 ($p>0.05$) BI with CTRL, since once ascorbic acid has probably completely oxidized to
472 dehydroascorbic acid, coloured quinones were formed again leading to browning
473 (Nicolas et al., 1994; Oms-Oliu et al., 2010).
474
475

Table 1. Browning index (BI), soluble solids content (SSC, %), titratable acidity (TA, g kg^{-1}) and pH of fresh−cut apples with different antibrowning dipping treatments during storage at 5 ºC (n=3±SD).

	Treatment	Storage time (days at 5 ºC)			
		0	3	6	9
BI	CTRL	$40.27 \pm 2.87^{A}_{b}$	$41.41 \pm 2.88^{AB}_{b}$	$50.44 \pm 0.53^{A}_{a}$	$53.26 \pm 2.70^{A}_{a}$
	L0.5	$35.34 \pm 0.71^{B}_{b}$	$39.89 \pm 2.53^{AB}_{b}$	$48.92 \pm 2.67^{AB}_{a}$	$51.79 \pm 0.88^{AB}_{a}$
	L1	$32.00 \pm 1.41^{B}_{c}$	$36.57 \pm 0.99^{B}_{b}$	$48.26 \pm 1.97^{AB}_{a}$	$49.29 \pm 1.65^{AB}_{a}$
	L2	$30.71 \pm 0.40^{B}_{b}$	$42.51 \pm 2.56^{AB}_{a}$	$45.54 \pm 1.50^{B}_{a}$	$43.81 \pm 2.56^{C}_{a}$
	AA	$33.32 \pm 2.31^{B}_{b}$	$44.99 \pm 3.01^{A}_{a}$	$49.79 \pm 1.50^{AB}_{a}$	$47.62 \pm 0.35^{BC}_{a}$
SSC	CTRL	$14.00 \pm 1.00^{A}_{a}$	$13.23 \pm 0.67^{A}_{a}$	$14.43 \pm 0.81^{A}_{a}$	$14.67 \pm 1.04^{A}_{a}$
	L0.5	$14.67 \pm 0.58^{A}_{a}$	$13.00 \pm 0.35^{A}_{b}$	$13.53 \pm 0.42^{A}_{ab}$	$13.73 \pm 0.64^{A}_{ab}$
	L1	$13.93 \pm 0.12^{A}_{a}$	$13.43 \pm 0.93^{A}_{a}$	$13.87 \pm 0.99^{A}_{a}$	$13.50 \pm 0.50^{A}_{a}$
	L2	$14.13 \pm 0.71^{A}_{a}$	$12.67 \pm 0.61^{A}_{a}$	$13.47 \pm 0.55^{A}_{a}$	$13.33 \pm 0.42^{A}_{a}$
	AA	$13.97 \pm 1.50^{A}_{a}$	$14.17 \pm 1.04^{A}_{a}$	$13.90 \pm 0.96^{A}_{a}$	$14.10 \pm 0.36^{A}_{a}$
TA	CTRL	$0.28 \pm 0.01^{AB}_{a}$	$0.32 \pm 0.04^{A}_{a}$	$0.29 \pm 0.05^{A}_{a}$	$0.34 \pm 0.04^{A}_{a}$
	L0.5	$0.32 \pm 0.02^{A}_{a}$	$0.24 \pm 0.03^{A}_{b}$	$0.27 \pm 0.01^{A}_{b}$	$0.24 \pm 0.01^{B}_{b}$
	L1	$0.23 \pm 0.04^{B}_{a}$	$0.23 \pm 0.03^{A}_{a}$	$0.25 \pm 0.02^{A}_{a}$	$0.28 \pm 0.02^{AB}_{a}$
	L2	$0.27 \pm 0.04^{AB}_{a}$	$0.26 \pm 0.02^{A}_{a}$	$0.26 \pm 0.01^{A}_{a}$	$0.25 \pm 0.03^{B}_{a}$
	AA	$0.26 \pm 0.02^{AB}_{a}$	$0.29 \pm 0.07^{A}_{a}$	$0.28 \pm 0.07^{A}_{a}$	$0.27 \pm 0.03^{AB}_{a}$
pH	CTRL	$4.00 \pm 0.10^{A}_{a}$	$4.07 \pm 0.06^{A}_{a}$	$4.17 \pm 0.32^{A}_{a}$	$3.83 \pm 0.25^{A}_{a}$
	L0.5	$4.07 \pm 0.06^{A}_{a}$	$4.20 \pm 0.17^{A}_{a}$	$4.33 \pm 0.21^{A}_{a}$	$3.97 \pm 0.06^{A}_{a}$
	L1	$4.10 \pm 0.10^{A}_{ab}$	$4.17 \pm 0.06^{A}_{a}$	$4.13 \pm 0.12^{A}_{ab}$	$3.93 \pm 0.06^{A}_{b}$
	L2	$4.13 \pm 0.15^{A}_{a}$	$4.00 \pm 0.00^{A}_{a}$	$4.07 \pm 0.06^{A}_{a}$	$3.93 \pm 0.06^{A}_{a}$
	AA	$4.13 \pm 0.06^{A}_{a}$	$3.97 \pm 0.06^{A}_{ab}$	$3.97 \pm 0.15^{A}_{ab}$	$3.87 \pm 0.06^{A}_{b}$

BI: LSD (A)***=1.66; LSD (B)***=1.48; LSD (A×B)***=3.32
SSC: LSD (A) ns; LSD (B)*=0.57; LSD (A×B) ns
TA: LSD (A)**=0.03; LSD (B) ns; LSD (A×B) ns
pH: LSD (A) ns; LSD (B)***=0.10; LSD (A×B) ns

The browning inhibition observed in those samples including lycopene microspheres may be due to the high antioxidant properties of this carotenoid (Egydio et al., 2010). Lycopene can chelate oxygen with a chelating constant two fold higher than β-carotene (Di Mascio et al., 1989; Vági et al., 2007). Additionally, lycopene can sequester other free radicals (NO2•, RS•, etc.) and protect DNA and cellular membranes from oxidative damage (Mortensen et al., 1997; Woodall et al., 1997). According to phenolic compounds' section, lycopene microspheres highly controlled the enzymatic oxidation of

491 phenolic compounds. Such fast antibrowning effect of lycopene microspheres just after
492 fresh−cut processing may be due to the controlled lycopene release from the microspheres
493 (Wu et al., 2013).
494 Sample browning increased during storage, showing CTRL samples the highest BI after
495 9 d at 5 ºC (Table 1). This high browning encourages the use of anti−browning dipping
496 treatments in fresh−cut apples as previously stated (Cortellino et al., 2015; Rojas-Graü et
497 al., 2009). The lowest browning increments during storage was observed in L2 samples
498 with a BI of 43.8 after 9 d. Meanwhile, the remaining treatments showed higher BI
499 (49.3−53.3), without significant ($p>0.05$) differences among them. In that sense, dipping
500 of fresh−cut apples in a solution containing 2 g L^{-1} of lycopene microspheres controlled
501 browning better than the lower lycopene microspheres concentrations or the ascorbic acid
502 treatment (commonly used by the fresh−cut industry).
503
504 **3.5. Physicochemical quality**
505 Physicochemical quality of apples is mainly defined by sugars and organic acids contents
506 (among other quality parameters such as firmness and aroma) that are reflected in a
507 balanced sweet and acid flavour, which is expected by the consumer. Acid and sweet
508 flavour scores from sensory analyses of apples have been highly correlated with TA and
509 SSC (Harker et al., 2002). On processing day, SSC and TA of CTRL samples were 13.7
510 % and 0.28 g kg^{-1}, respectively (Table 1). The dipping treatments did not induce SSC
511 changes on processing day while TA changes were < 0.1 g kg^{-1}. Storage time factor did
512 not affect SSC and TA (Table 1). The minimum SSC and TA changes to be detected after
513 consumption of apples are 1 % and 0.8 g kg^{-1}, respectively (Harker et al., 2002). Then,
514 dipping of fresh−cut apples in the solution containing lycopene microspheres did not

515 induce remarkable physicochemical changes on processing day nor during storage,

516 ensuring their consumer acceptance at least for 9 d at 5 ºC.

517

518 **3.6. Microbial quality**

519 Samples showed an excellent microbial quality on processing day with low psychrophilic

520 (1.8 log CFU g^{-1}) and Y+M counts (2.4 log CFU g^{-1}), while mesophilic and

521 *Enterobacteria* remained below the detection limit (1 log CFU g^{-1}) (Table 2). No

522 presence of of *Salmonella* spp., *Listeria monocytogenes* and generic *Escherichia coli* was

523 detected during storage of samples (data not shown). In general, microbial quality was

524 not highly affected (< 0.8 log units) after incorporation of lycopene microspheres being

525 the registered low increments due to the microbial contamination from the used wall

526 materials (gum Arabic and gelatine).

527

528 **Table 2.** Microbial loads (log CFU g^{-1}) of fresh−cut apples with different antibrowning

529 dipping treatments during storage at 5 ºC (n=3±SD).

	Treatment	Storage time (days at 5 ºC)			
		0	3	6	9
Mesophiles	CTRL	$< DL$	$1.69 \pm 0.27^{AB}_{ab}$	$1.38 \pm 0.43^{B}_{b}$	$2.42 \pm 0.49^{A}_{a}$
	L0.5	$1.20 \pm 0.35^{A}_{b}$	$2.30 \pm 0.41^{A}_{ab}$	$2.45 \pm 0.18^{A}_{a}$	$2.19 \pm 0.36^{A}_{a}$
	L1	$< DL$	$1.67 \pm 0.49^{AB}_{ab}$	$2.33 \pm 0.61^{AB}_{a}$	$2.13 \pm 0.15^{AB}_{a}$
	L2	$< DL$	$1.16 \pm 0.15^{B}_{b}$	$2.05 \pm 0.34^{AB}_{a}$	$1.55 \pm 0.49^{AB}_{ab}$
	AA	$< DL$	$< DL$	$1.41 \pm 0.36^{AB}_{a}$	$1.10 \pm 0.17^{B}_{a}$
Psychrophiles	CTRL	$1.76 \pm 0.45^{AB}_{a}$	$2.19 \pm 0.24^{AB}_{a}$	$2.34 \pm 0.51^{AB}_{a}$	$2,60 \pm 0.25^{AB}_{a}$
	L0.5	$2.29 \pm 0.40^{AB}_{a}$	$2.12 \pm 0.18^{AB}_{a}$	$2.09 \pm 0.61^{AB}_{a}$	$2.70 \pm 0.26^{AB}_{a}$
	L1	$2.89 \pm 0.43^{A}_{a}$	$2.14 \pm 0.47^{A}_{a}$	$3.19 \pm 0.49^{A}_{a}$	$2.65 \pm 0.45^{A}_{a}$
	L2	$1.76 \pm 0.73^{B}_{a}$	$2.24 \pm 0.68^{B}_{a}$	$1.82 \pm 0.72^{B}_{a}$	$2.33 \pm 0.52^{B}_{a}$
	AA	$1.62 \pm 0.25^{B}_{a}$	$1.54 \pm 0.47^{B}_{a}$	$2.37 \pm 0.37^{B}_{a}$	$2.32 \pm 0.75^{B}_{a}$
Enterobacteria	CTRL	$< DL$	$1.16 \pm 0.28^{A}_{ab}$	$1.30 \pm 0.52^{A}_{a}$	$1.57 \pm 0.56^{A}_{a}$
	L0.5	$< DL$	$1.29 \pm 0.36^{A}_{ab}$	$1.53 \pm 0.47^{A}_{a}$	$1.32 \pm 0.28^{A}_{a}$
	L1	$< DL$	$1.26 \pm 0.24^{A}_{ab}$	$1.53 \pm 0.47^{A}_{a}$	$1.32 \pm 0.28^{A}_{a}$
	L2	$< DL$	$1.20 \pm 0.34^{A}_{ab}$	$1.40 \pm 0.49^{A}_{a}$	$1.45 \pm 0.48^{A}_{a}$
	AA	$< DL$	$< DL$	$1.13 \pm 0.16^{A}_{a}$	$1.23 \pm 0.40^{A}_{a}$

Yeast and moulds	CTRL	$2.40 \pm 0.17^{A}_{b}$	$2.53 \pm 0.21^{A}_{b}$	$2.19 \pm 0.20^{B}_{b}$	$3.33 \pm 0.47^{AB}_{a}$
	L0.5	$2.40 \pm 0.17^{A}_{b}$	$2.73 \pm 0.26^{A}_{b}$	$2.82 \pm 0.45^{AB}_{ab}$	$3.57 \pm 0.28^{A}_{a}$
	L1	$2.40 \pm 0.17^{A}_{b}$	$2.96 \pm 0.42^{A}_{ab}$	$3.42 \pm 0.45^{A}_{a}$	$3.05 \pm 0.06^{AB}_{ab}$
	L2	$2.30 \pm 0.00^{A}_{a}$	$2.40 \pm 0.17^{A}_{a}$	$2.74 \pm 0.40^{AB}_{a}$	$2.66 \pm 0.19^{B}_{a}$
	AA	$2.36 \pm 0.10^{A}_{b}$	$2.46 \pm 0.15^{A}_{b}$	$3.31 \pm 0.25^{A}_{a}$	$3.17 \pm 0.23^{AB}_{a}$

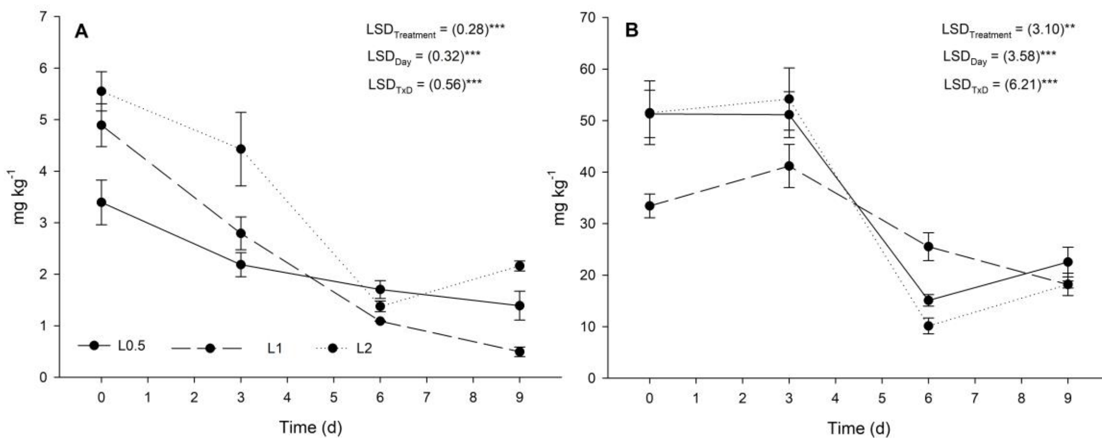
Mesophiles: LSD (A)***=0.27; LSD (B)***=0.24; LSD (A×B)**=0.54
Psychrophiles: LSD (A)**=0.41; LSD (B)*=0.36; LSD (A×B) ns
Enterobacteria: LSD (A) ns; LSD (B)**=0.26; LSD (A×B) ns
Yeast and moulds: LSD (A)**=0.23; LSD (B)***=0.20; LSD (A×B) **=0.46
DL: detection limit (1 log CFU g^{-1})

Microbial loads of CTRL samples showed mesophilic and Y+M increments of 1.4 and 0.9 log units, respectively, after 9 d at 5 ºC while the rest of the microbial groups remained unchanged. Psychrophiles and *Enterobacteria* loads were not changed ($p>0.05$) during storage. On the other side, the incorporation of lycopene microspheres at the highest concentration (L2) controlled all microbial groups with unchanged ($p>0.05$) microbial loads after 9 d. As expected, microbial growth was also controlled with the AA treatment remaining all microbial groups unchanged, except Y+M that increased by 0.8 log units after 9 d. Antimicrobial properties of ascorbic acid dipping treatments in fresh−cut apples, and other fresh−cut fruit and vegetables, are well−known due to the pH decrease. The observed antimicrobial effect of lycopene microspheres at 2 g L^{-1} may be owed to the antimicrobial properties of lycopene as previously observed (Al-Oqaili et al., 2011; Dhanawade and Sakhare, 2014). Accordingly, fresh−cut apples with lower concentrations of lycopene microspheres (L0.5 and L1) registered mesophilic and Y+M increments of ≈1 log unit after 9 d.

In conclusion, incorporation of lycopene microspheres at 2 g L^{-1} controlled microbial growth in fresh−cut apples during storage up to 9 d at 5 ºC.

3.7. Lycopene

556 Fortification of fresh−cut apples with the lycopene microspheres led to total cis and 557 all−trans lycopene isomer contents of 33.4−51.5 and 3.4−5.6 mg kg^{-1}, respectively 558 (Figure 4). As expected, the higher the microspheres concentration added, the higher the 559 lycopene content. Cis−lycopene isomers of all samples remained unchanged during the 560 first 3 d of storage. On the other side, all−trans contents of L0.5 and L1 samples decreased 561 by 36−43 % after 3 d. The all−trans reduction observed in L0.5/L1 samples was ≈2−fold 562 lower in L2 samples on day 3. Such higher initial lycopene stability from L2 samples may 563 be owed to a lower lycopene oxidation due to the higher total antioxidant capacity from 564 these samples (see total antioxidant capacity section). A general lycopene decrease was 565 observed from day 3 to day 9 with total cis and all–trans lycopene isomer contents of 566 10.1−25.5 and 1.1−1.7 mg kg^{-1}, respectively, on day 6. Overall, cis−lycopene isomers, 567 the lycopene isomers of bioactive interest, were the predominant lycopene isomers during 568 storage of fresh−cut apples with these microspheres still reaching cis−lycopene contents 569 of ≈20 mg kg^{-1} after 9 d of storage.



570

571 **Figure 4.** Contents of lycopene isomers (A, all−trans; B, sum of cis−lycopene isomers) 572 (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments during 573 storage at 5 ºC (n=3±SD).

574

575 Conclusively, incorporation of lycopene microspheres led to a lycopene enrichment of
576 fresh−cut apples, a fruit with negligible contents of this carotenoid (Delgado-Pelayo et
577 al., 2014). A daily lycopene intake of 5–10 mg has been recommended to maintain
578 required serum lycopene levels and to reduce lipid peroxidation (Rao and Shen, 2002). In
579 this sense, 200 g of fresh−cut apples would provide between 1 (day 9) and 1.6 (day 0) mg
580 of total (trans+cis) lycopene, highly contributing to the recommended daily intake of this
581 carotenoid. Cis–lycopene isomers are more easily taken up by mixed micelles in the
582 intestine being more bioavailable compared with all–trans isomers (Boileau et al., 1999).
583 Therefore, the high cis−isomer proportion (10–15–fold higher than all–trans form) in the
584 fresh−cut apples with lycopene microspheres would also lead to a higher lycopene
585 bioavailability with a more efficient and higher contribution to the recommended daily
586 intake of lycopene.

587

588 **3.8. Phenolic compounds and total antioxidant capacity**

589 Phenolic content has been highly correlated to TAC in apples due to the high phenolic
590 content of this fruit, while low or undetected levels of other antioxidants like vitamin C
591 (120.4 mg kg^{-1}) or carotenoids have been reported (Aguayo et al., 2015; Gardner et al.,
592 2000; Lee and Kader, 2000). Among the three TAC methods, FRAP showed the highest
593 TPC–TAC correlation (R^2=0.89), while DPPH and ABTS showed lower correlations
594 (R^2=0.49 and 0.23, respectively) (data not shown). Thus, FRAP data is shown in Table 3
595 while DPPH and ABTS are supplied as additional data in the Suplementary material 3.

596

597 **Table 3.** Total phenolic content (TPC) (mg kg^{-1}) and total antioxidant capacity (TAC)
598 (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments during
599 storage at 5 ºC (n=3±SD).

	Treatment	Storage time (days at 5 ºC) 0	3	6	9
TPC	CTRL	$266.4 \pm 94.9^{B}_{a}$	$355.7 \pm 23.6^{B}_{a}$	$359.4 \pm 51.5^{B}_{a}$	$405,8 \pm 32,8^{A}_{a}$
	L0.5	$259.5 \pm 17.3^{B}_{c}$	$311.7 \pm 17.2^{BC}_{bc}$	$341.8 \pm 20.1^{B}_{ab}$	$383.9 \pm 40.0^{AB}_{a}$
	L1	$379.8 \pm 83.3^{B}_{ab}$	$271.1 \pm 18.3^{C}_{b}$	$435.2 \pm 49.2^{B}_{a}$	$300.1 \pm 60.5^{B}_{ab}$
	L2	$339.4 \pm 34.5^{B}_{ab}$	$362.3 \pm 31.4^{AB}_{ab}$	$399.7 \pm 31.6^{B}_{a}$	$299.2 \pm 20.8^{B}_{b}$
	AA	$594.0 \pm 71.0^{A}_{a}$	$419.1 \pm 12.1^{A}_{b}$	$575.4 \pm 71.4^{A}_{a}$	$480.2 \pm 15.3^{A}_{ab}$
TAC	CTRL	$694.7 \pm 138.7^{C}_{c}$	$949.6 \pm 52.3^{AB}_{ab}$	$911.4 \pm 69.9^{BC}_{bc}$	$1180.8 \pm 87.0^{B}_{a}$
	L0.5	$727.3 \pm 20.4^{C}_{b}$	$738.6 \pm 70.6^{C}_{b}$	$866.9 \pm 82.2^{C}_{ab}$	$942.4 \pm 102.9^{BC}_{a}$
	L1	$940.3 \pm 157.0^{BC}_{ab}$	$758.5 \pm 97.3^{BC}_{b}$	$1201.3 \pm 111.7^{B}_{a}$	$860.9 \pm 208.9^{BC}_{ab}$
	L2	$1179.2 \pm 57.8^{B}_{a}$	$1055.7 \pm 68.3^{A}_{a}$	$1026.8 \pm 41.1^{BC}_{a}$	$824.0 \pm 101.4^{C}_{b}$
	AA	$1734.5 \pm 77.2^{A}_{a}$	$1091.3 \pm 80.3^{A}_{b}$	$1636.6 \pm 202.6^{A}_{a}$	$1533.6 \pm 66.0^{A}_{a}$

TPC: LSD (A)***=38.42; LSD (B)***=34.36; LSD (A×B)***=76.84
TAC (by FRAP method): LSD (A)***=87.55; LSD (B)***=78.31; LSD (A×B)***=175.10

Supplementary material 3. Total antioxidant capacity (TAC; by two methods: DPPH and ABTS) (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments during storage at 5 ºC (n=3±SD).

TAC	Treatment	Storage time (days at 5 ºC) 0	3	6	9
ABTS	CTRL	$1494.9 \pm 80.5^{C}_{b}$	$2039.8 \pm 99.9^{A}_{a}$	$1818.9 \pm 97.8^{A}_{a}$	$1150.0 \pm 164.2^{A}_{c}$
	L0.5	$1428.3 \pm 48.9^{C}_{ab}$	$1616.1 \pm 43.2^{B}_{a}$	$1325.1 \pm 194.1^{B}_{b}$	$1205.8 \pm 52.3^{A}_{b}$
	L1	$1426.1 \pm 243.9^{C}_{b}$	$1571.2 \pm 9.3^{B}_{b}$	$2071.4 \pm 108.0^{A}_{a}$	$932.9 \pm 10.6^{B}_{c}$
	L2	$2038.7 \pm 157.9^{B}_{a}$	$1811.7 \pm 210.1^{AB}_{a}$	$1723.2 \pm 192.4^{AB}_{a}$	$683.0 \pm 3.0^{C}_{b}$
	AA	$2711.8 \pm 109.0^{A}_{a}$	$1677.5 \pm 66.4^{B}_{b}$	$1875.9 \pm 215.9^{A}_{b}$	$1273.0 \pm 14.1^{A}_{c}$
DPPH	CTRL	$694.6 \pm 105.8^{C}_{ab}$	$839.8 \pm 55.7^{C}_{a}$	$860.8 \pm 26.8^{BC}_{a}$	$589.7 \pm 67.0^{B}_{b}$
	L0.5	$527.8 \pm 28.0^{D}_{b}$	$642.1 \pm 52.0^{D}_{ab}$	$699.5 \pm 97.0^{C}_{a}$	$731.6 \pm 27.2^{B}_{a}$
	L1	$543.9 \pm 4.9^{CD}_{c}$	$672.0 \pm 20.5^{D}_{b}$	$883.2 \pm 23.9^{B}_{a}$	$586.2 \pm 84.0^{B}_{bc}$
	L2	$871.1 \pm 20.4^{B}_{b}$	$1086.8 \pm 36.0^{B}_{a}$	$1163.3 \pm 85.6^{A}_{a}$	$693.8 \pm 28.1^{B}_{c}$
	AA	$1304.6 \pm 70.9^{A}_{ab}$	$1330.1 \pm 61.5^{A}_{a}$	$1167.7 \pm 17.1^{A}_{b}$	$928.0 \pm 62.6^{A}_{c}$

ABTS: LSD (A)***=107.29; LSD (B)***=95.97; LSD (A×B)***=214.59
DPPH: LSD (A)***=46.70; LSD (B)***=41.77; LSD (A×B)***=93.39

Chlorogenic acid, (–)epicatechin and procyanidins B1 and B2 were the major identified phenolic compounds with contents of 133.2, 26.7 and 10.2/10.8 mg kg^{-1}, respectively (Table 4) (as previously reported for Braeburn apple (Rössle et al., 2010)) accounting their sum the 70 % of TPC. The higher TPC compared to the sum of individual phenolic

compounds may be owed to other antioxidant compounds (vitamin C, etc.) from apples, which may also react with the Folin–Ciocalteu reagent used for the TPC analysis. Among minor identified compounds were caffeic acid (0.83 mg kg^{-1}), phloridzidin (0.52 mg kg^{-1}), *p*–coumaric acid (0.17 mg kg^{-1}) and ferulic acid (0.14 mg kg^{-1}) (data not shown), which were not affected (by either treatments, storage time or their interaction.

Table 4. Individual phenolic compounds (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments during storage at 5 ºC (n=3±SD).

		Storage time (days at 5 ºC)			
Individual phenols	Treatment	0	3	6	9
Chlorogenic acid	CTRL	$133.2 \pm 12.3^{C}_{a}$	$120.1 \pm 37.1^{B}_{a}$	$160.3 \pm 33.5^{B}_{a}$	$195.6 \pm 48.3^{B}_{a}$
	L0.5	$181.9 \pm 8.6^{B}_{a}$	$195.8 \pm 16.7^{A}_{a}$	$184.3 \pm 21.6^{B}_{a}$	$183.2 \pm 29.7^{B}_{a}$
	L1	$198.1 \pm 17.6^{B}_{b}$	$230.4 \pm 33.4^{A}_{b}$	$186.9 \pm 7.8^{B}_{b}$	$309.7 \pm 9.0^{A}_{a}$
	L2	$207.9 \pm 8.7^{B}_{b}$	$232.6 \pm 13.9^{A}_{ab}$	$251.1 \pm 8.6^{A}_{a}$	$215.5 \pm 24.7^{B}_{ab}$
	AA	$284.9 \pm 7.6^{A}_{a}$	$211.2 \pm 17.7^{A}_{b}$	$202.6 \pm 10.4^{AB}_{b}$	$232.0 \pm 24.2^{AB}_{b}$
Epicatechin	CTRL	$26.7 \pm 4.8^{D}_{a}$	$25.8 \pm 6.5^{B}_{a}$	$39.5 \pm 5.9^{AB}_{a}$	$39.1 \pm 4.3^{BC}_{a}$
	L0.5	$35.9 \pm 1.0^{CD}_{a}$	$39.8 \pm 4.1^{A}_{a}$	$35.9 \pm 4.3^{B}_{a}$	$34.1 \pm 4.2^{C}_{a}$
	L1	$44.5 \pm 5.0^{BC}_{ab}$	$40.8 \pm 6.2^{A}_{ab}$	$34.6 \pm 1.4^{B}_{b}$	$45.8 \pm 2.8^{AB}_{a}$
	L2	$48.8 \pm 2.2^{AB}_{a}$	$47.4 \pm 4.5^{A}_{a}$	$48.4 \pm 3.7^{A}_{a}$	$43.2 \pm 4.0^{BC}_{a}$
	AA	$55.9 \pm 4.5^{A}_{a}$	$42.0 \pm 3.4^{A}_{b}$	$40.3 \pm 2.4^{AB}_{b}$	$54.4 \pm 4.8^{A}_{a}$
Catechin	CTRL	$4.5 \pm 0.8^{B}_{a}$	$3.0 \pm 0.9^{C}_{a}$	$3.8 \pm 0.2^{A}_{a}$	$3.8 \pm 0.8^{B}_{a}$
	L0.5	$4.1 \pm 0.5^{B}_{b}$	$4.9 \pm 0.6^{B}_{ab}$	$4.5 \pm 0.8^{A}_{ab}$	$5.9 \pm 0.7^{A}_{a}$
	L1	$6.8 \pm 0.5^{A}_{a}$	$5.2 \pm 0.4^{B}_{b}$	$3.9 \pm 0.6^{A}_{c}$	$5.7 \pm 0.5^{A}_{ab}$
	L2	$4.1 \pm 0.2^{B}_{b}$	$5.5 \pm 0.6^{B}_{a}$	$4.7 \pm 0.7^{A}_{ab}$	$3.9 \pm 0.3^{B}_{b}$
	AA	$4.6 \pm 0.6^{B}_{b}$	$7.8 \pm 0.4^{A}_{a}$	$5.0 \pm 0.6^{A}_{b}$	$4.5 \pm 0.4^{AB}_{b}$
Caffeic acid	CTRL	$0.8 \pm 0.1^{AB}_{a}$	$0.8 \pm 0.1^{A}_{a}$	$0.9 \pm 0.1^{A}_{a}$	$1.1 \pm 0.2^{A}_{a}$
	L0.5	$0.8 \pm 0.1^{B}_{a}$	$0.8 \pm 0.1^{A}_{a}$	$0.9 \pm 0.1^{A}_{a}$	$0.8 \pm 0.1^{B}_{a}$
	L1	$0.7 \pm 0.1^{B}_{c}$	$0.8 \pm 0.1^{A}_{bc}$	$1.1 \pm 0.0^{A}_{a}$	$0.9 \pm 0.0^{AB}_{ab}$
	L2	$1.1 \pm 0.0^{A}_{a}$	$0.8 \pm 0.2^{A}_{a}$	$0.9 \pm 0.1^{A}_{a}$	$0.9 \pm 0.1^{AB}_{a}$
	AA	$1.1 \pm 0.1^{A}_{a}$	$0.7 \pm 0.2^{A}_{b}$	$1.0 \pm 0.1^{A}_{ab}$	$0.9 \pm 0.1^{AB}_{ab}$

Chlorogenic acid: LSD (A)***=18.69; LSD (B)**=16.72; LSD (A×B)***=37.39
Epicatechin: LSD (A)***=3.51; LSD (B)*=3.14; LSD (A×B)***=7.02
Catequin: LSD (A)***=0.48; LSD (B)**=0.43; LSD (A×B)***=0.95
Caffeic acid: LSD (A) ns; LSD (B)**= 0.09; LSD (A×B)***=0.19

The TPC of samples ranged between 259.5 and 379.8 mg kg^{-1} on processing day, without differences among them, except AA samples that showed the highest TPC of 594.0 mg

632 kg^{-1} (Table 3). Similarly, AA samples showed the highest contents of chlorogenic acid,
633 the major phenolic compound, on processing day. The latter finding may be explained
634 since ascorbic acid from AA treatment protected phenolic compounds from oxidation.
635 Nevertheless, such ascorbic acid protection was reduced during storage showing AA
636 samples similar TPC levels to CTRL after 9 d at 5 ºC. The ascorbic acid degradation
637 through time, even at low storage temperatures, has been widely reported in literature
638 (Castillejo et al., 2017; Lee and Kader, 2000).
639 Chlorogenic acid content was enhanced by 21–56 % in L1 and L2 samples after 6–9 d.
640 Nevertheless, chlorogenic acid remained unchanged in CTRL and L0.5 samples during
641 storage. Wounding, as occurred during fresh−cut processing (cuting), has been
642 considered as an abiotic stress able to enhance the phenylalanine ammonia lyase activity,
643 the key enzyme in the phenolic byosinthesis pathway (Cisneros-Zevallos, 2003; Formica-
644 Oliveira et al., 2016). As a result, the stress–synthesized phenolic compounds were
645 protected from oxidation due to the released lycopene from microspheres with higher
646 lycopene contents (L1 and L2), while stress–synthesized phenolic compounds were
647 degraded for the remaining samples. The previous high phenolic enhancements were not
648 observed for the rest of phenolic compounds, which levels remained unchaged or even
649 decreased (with reductions lower than 15 %) after 9 d at 5 ºC. Similarly to our data, the
650 highest phenolic enhancements after wounding of carrots were observed for chlorogenic
651 acid, the major phenolic compound in carrots, showing the remaining phenolic
652 compounds lower or unchanged levels (Formica-Oliveira et al., 2016; Jacobo-Velázquez
653 et al., 2011). The latter finding could be owed to a level–dependent and/or phenolic
654 specificity for such stress–enhancement of chlorogenic acid. Contrary to individual
655 phenolics, L1 and L2 samples showed the lowest TPC after 9 d (Table 3). Other
656 antioxidant compounds, like carotenoids (i.e. lycopene, β-carotene), may also react with

657 the Folin–Ciocalteu reagent. In that sense, while L1 and L2 showed high TPC on day 0,
658 when lycopene was added to apples, the lycopene degradation of samples during storage
659 (Figure 4) led to the low TPC of L1 and L2 after 9 d. In that sense, it is crucial to jointly
660 analyze TPC with individual phenols to better study overestimations with the Folin–
661 Ciocalteu method for TPC analysis.
662

663 ## 4. CONCLUSIONS

664 Tomato by-products from the food industry can be revalorized due to its high lycopene
665 content of the skin. Lycopene thermal extraction, combined with TiO_2 nanoparticles, of
666 the tomato skin allowed to reach an excellent lycopene extraction yield, with
667 predominance of cis−lycopene isomers. The fresh−cut apples fortified with the obtained
668 lycopene microspheres controlled the enzymatic browning after 9 d at 5 ºC, without
669 affecting the physicochemical or microbial quality. Furthermore, the incorporation of
670 lycopene microspheres increased the health−promoting properties of fresh−cut apples
671 together with enhancement of phenolic compounds up to 56 % (for chlorogenic acid) after
672 9 d at 5 ºC. In future experiments, it would be interesting to optimize lycopene
673 encapsulation from tomato waste from food industry and observe the effects of them on
674 the fresh−cut apples quality.
675

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925 **FIGURE AND TABLE CAPTIONS**

926

927 **Figure 1.** Contents of lycopene isomers (g kg^{-1}) in tomato skin.

928

929 **Figure 2.** Contents of lycopene isomers (g kg^{-1}) extracted from tomato skin using
930 different extraction treatments.

931

932 **Figure 3.** Image obtained by optical microscopy of lycopene microspheres. W: wall
933 material; C: core material.

934

935 **Figure 4.** Contents of lycopene isomers (A, all−trans; B, sum of cis−lycopene isomers)
936 (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments during
937 storage at 5 ºC (n=3±SD).

938

939 **Table 1.** Browning index (BI), soluble solids content (SSC, %), titratable acidity (TA, g
940 kg^{-1}) and pH of fresh−cut apples with different antibrowning dipping treatments during
941 storage at 5 ºC (n=3±SD).

942

943 **Table 2.** Microbial loads (log CFU g^{-1}) of fresh−cut apples with different antibrowning
944 dipping treatments during storage at 5 ºC (n=3±SD).

945

946 **Table 3.** Total phenolic content (TPC) (mg kg^{-1}) and total antioxidant capacity (TAC)
947 (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments during
948 storage at 5 ºC (n=3±SD).

949

950 **Table 4.** Individual phenolic compounds (mg kg^{-1}) of fresh−cut apples with different

951 antibrowning dipping treatments during storage at 5 ºC (n=3±SD).

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953 **SUPPLEMENTARY MATERIAL**

954

955 **Supplementary material 1.** TEM images of TiO_2 nanoparticles.

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957 **Supplementary material 2.** XRD patterns of TiO_2 nanoparticles.

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959 **Supplementary material 3**. Total antioxidant capacity (TAC; by two methods: DPPH
960 and ABTS) (mg kg^{-1}) of fresh−cut apples with different antibrowning dipping treatments
961 during storage at 5 ºC (n=3±SD).