Ferulic acid application to control growth *Listeria monocytogenes* and *Salmonella enterica* on fresh-cut apples and melon, and its effect in quality parameters.

Authors: Iolanda Nicolau-Lapeña[1], Ingrid Aguiló-Aguayo[2], Gloria Bobo[2], Inmaculada Viñas[1], Marina Anguera[2], Maribel Abadias[2]*.

[1] Food Technology Department, University of Lleida, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Catalonia, Spain.

[2] IRTA, Postharvest Programme, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, Edifici Fruitcentre, 25003, Lleida, Catalonia, Spain.

*Corresponding author:

Maribel Abadias: isabel.abadias@irta.cat

Abbreviations

AAE, ascorbic acid equivalents; BI, browning index; CFU, colony forming units; CT, control treatment; W, water control; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FA, ferulic acid; DRBC, dichloran rose bengale chloramphenicol agar FRAP, ferric reducing antioxidant power; FW, fresh weight; GAE, gallic acid equivalents; NS, NatureSeal ®; PCA, plate count agar; TA, titratable acidity; TAM, total aerobic mesophylls; TCD, total color difference; TPC, total phenolic content; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine; TSA, triptone soy agar; TSB, triptone soy broth; TSS, total soluble solids; UC, untreated control; XLD, xilose lysine deoxycholate agar; Y&M, yeast and mould

Abstract

Listeria monocytogenes can grow under conditions at which fresh-cut fruit are stored, whereas *Salmonella* spp. has been associated with a number of outbreaks related to such products. It is therefore necessary to find products capable of reducing microbial counts while maintaining quality of the product. In this regard, ferulic acid (FA) has shown antimicrobial, antioxidant and many physiological functions in humans. This study aimed to test the efficacy of FA in fresh-cut apple and melon in two ways: (a) to prevent pathogenic growth and (b) to maintain fruit quality during storage, maintaining color and preventing enzymatic browning. For this purpose, of *L. monocytogenes* (3 strains) and *S. enterica* (4 strains) were inoculated in both fruits. FA at concentrations ranging from 2.5 to 15 g L^{-1} were tested against individual strains and the results showed that FA did not have any bactericidal effect after application. FA effect was observed at the end of the storage (7 d, 10 ºC) with higher effect against *L. monocytogenes* (averaging 4.2±0.7 log CFU g^{-1}) than against *S. enterica* (averaging 1.9±1.3 log CFU g^{-1}). The reductions were significantly different from the samples without FA, but significant differences were not found among the 3 tested concentrations. Comparison between immersion and spray applications of FA revealed that immersion was the best method. When the effect of the selected FA dose on quality was evaluated, we found that FA did not prevent the increase of browning index in apples. However, melon treated samples did not overcome significant colour changes during storage at 4 ºC. FA did not inhibit the growth of total aerobic mesophylls and yeasts and molds, but maintained overall quality of the fruits, including pH, total soluble solids and titratable acidity. Overall, FA could be used in fresh-cut apple and melon to prevent growth of *L. monocytogenes* without affecting physicochemical quality, delivering a product with increased antioxidant activity and providing a new source of FA (0.25±0.04 g kg^{-1} of apple, and 1.22±0.07 g kg^{-1} of melon, dry weight basis).

Keywords:

Antimicrobial, antioxidant, shelf-life, pathogens, fruit, anti-browning.

1. Introduction

The International Fresh-cut Produce Association defines fresh-cut produce as "any fruit, vegetable or their combination subjected to a physical alteration from its original form, remaining in a fresh state" (Grau Rojas et al., 2010). Due to the higher demand for sustainable, fresh and healthy products, the fruit processing industry is experiencing an expanding period (Qadri et al., 2015). However, consumption of minimally processed fruits has been linked to several outbreaks of foodborne pathogens (Pinela and Ferreira, 2015). Growth of *Listeria monocytogenes, Salmonella* spp. and *Escherichia coli* O157:H7 has been previously confirmed on fresh-cut apples (Abadias et al., 2011) and melon (Abadias et al., 2012). Moreover, *Salmonella* spp. and *L. monocytogenes* have been related to several outbreaks related to the consumption of apples and melons (CDCP, 2014; Callejón et al., 2015). Furthermore, fresh-cut fruits' shelf-life tends to be short, mainly due to browning, loss of weight and loss of firmness (Wilson et al., 2019).

Preservatives are employed to inhibit microbial growth or to delay browning and ripening processes, critical factors to maintain consumer's safety and extend product shelf. Lately, with the emergence of bacteria resistance to chemical antibiotics, and the increasing mistrust of consumers towards chemical additives, there is a trend in the search for natural products with antioxidant and antimicrobial properties (Pernin et al., 2019b).

Ferulic acid (FA, [E]-3-[4-hydroxy-3-methoxy-phenyl] prop-2-enoic acid) is an ubiquitous phytochemical phenolic acid, the most common of the cinnamic acid group (Mattila and Kumpulainen, 2002). FA is an important structural component in the plant cell wall and serves to enhance its rigidity and strength (Kumar and Pruthi, 2014). The use of FA is approved as an antioxidant food additive in Japan, while natural extracts with high contents of FA are permitted in the US and most European countries to prevent lipid peroxidation of foods (Quitmann et al., 2014). Previously, we found that the half-inhibitory concentration (IC_{50}) of FA as antioxidant in *in vitro* trials was 0.45 g L^{-1} (Nicolau-Lapeña, 2021). Moreover, FA has been reported to have antimicrobial properties (Kumar and Pruthi, 2014). Its mode of action consists of making irreversible changes to membrane properties, including charge, intra and extracellular

77 permeability, and its physicochemical properties (Borges et al., 2013). Low minimum inhibitory
78 concentrations against several pathogenic bacteria have been elucidated for this compound
79 (Pacheco-Ordaz et al., 2017). Preliminary work has shown that the minimum inhibitory
80 concentration of this compound (tested *in vitro)* against 13 strains (belonging to 7 different
81 species) of food-borne pathogenic bacteria (including *Salmonella* spp., *Enterobacter aerogenes,*
82 *L. monocytogenes, Staphylococcus aureus,* and *E. coli*) ranged from 1.7 to 3.3 g FA L^{-1} (Nicolau-
83 Lapeña, 2021).

84 The objectives of this study were (i) to evaluate the effect of FA at different concentrations
85 (ranging from 1.0 to 15.0 g L^{-1}) in controlling growth of *S. enterica* and *L. monocytogenes* on
86 fresh-cut apple and melon stored at 4 ºC, (ii) to determine which application method (immersion
87 or spray) provides the highest effect and (iii) to study its action as an antioxidant agent in fresh-
88 cut apple and melon in order to delay browning or changes in colour, and its effect on other quality
89 parameters, including pH, total soluble content, titratable acidity, total phenolic content,
90 antioxidant capacity, firmness, and spoilage microbiota.

2. Materials and methods

2.1. Materials

Apple ('Golden Delicious') and melon ('Piel de sapo') fruits were obtained from local providers (Plusfresc, Spain). *Trans*-ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid, ≥ 99 %, W518301) was purchased from Sigma-Aldrich (Steinheim, Germany), and NatureSeal® was from Agricoat NatureSeal Ltd (Hungerford, United Kingdom).

The bacterial strains used comprised the serovars of *Salmonella enterica* subsp. *enterica*: Agona (ATCC BAA-707), Montevideo (ATCC BAA-710), Gaminara (ATCC BAA-711) and Typhimurium (CECT-4594) and *L. monocytogenes* serovar 1/2 (CECT-4031), serovar 4b (CECT-935) and serovar 1/2a, isolated from lettuce in our laboratory (Abadias et al., 2008).

Dey-Engley broth was purchased from Honeywell Fluka (Madrid, Spain). Tryptone soy broth (TSB), tryptone soy agar (TSA), yeast extract (YE), Palcam base agar and Palcam selective supplement for *Listeria*, potassium bisulfate, sodium chloride, xylose lysine deoxycholate agar (XLD), plate count agar (PCA), dichloran rose bengale chloramphenicol agar (DRBC), and peptone were acquired from Biokar Diagnostics (Allonne, France).

Ascorbic acid, gallic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and sodium carbonate, were obtained from Sigma-Aldrich (Steinheim, Germany). Methanol, ethanol, hydrochloric acid (37 %), sodium acetate, sodium hydroxide, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were purchased from Panreac (Llinars del Valles, Spain).

2.2. Methodology

2.2.1. Inoculum preparation

S. enterica strains were grown in 0.05 L of TSB, and *L. monocytogenes* strains in TSB supplemented with 6 g L^{-1} of yeast extract, 2.5 g L^{-1} glucose and 2.5 g L^{-1} K_2HPO_4 (TSBYE) for 24 h at 37 ºC, until stationary phase. Culture was centrifuged at 9800 × *g* for 10 min at 10 ºC. The pellet containing the bacteria was resuspended in 0.025 L saline solution (NaCl, 8.5 g L^{-1}). The population of bacterial suspensions was determined by plating in TSA and XLD, or TSA supplemented with 6 g L^{-1} of yeast extract, 2.5 g L^{-1} glucose and 2.5 g L^{-1} K_2HPO_4 (TSAYE) and Palcam, respectively, and incubated for 24 h at 37 ºC.

2.2.2. Preparation and inoculation of apple and melon

Prior to disinfection with ethanol 70 %, fruits were rinsed with tap water. Fruits were peeled and flesh cylinders of 1.2 cm Ø were taken off using a core borer and cut in 1.0 cm high with a sharp knife. Apple discs from several apples and melon were randomly distributed among treatments. Fruit discs were inoculated by immersion in a previously prepared suspension containing about 1.5×10^7 CFU mL^{-1} of each strain. Concentration was checked by serially diluting in saline peptone (SP, peptone 1 g L^{-1} and NaCl, 8.5 g L^{-1}) and plating in TSA for *S. enterica* or TSAYE for *L. monocytogenes* and incubated 24 h at 37 ºC. A ratio 1:10 (fruit:inoculum volume) was used for the inoculation, to assure complete immersion of all pieces. After thorough agitation for 2 min, fruit pieces were dried over a lab rack in a biosafety level 2 laminar flow cabinet.

2.2.3. *S. enterica* and *L.monocytogenes* determination

For bacterial counts, each disc was considered a repetition. Three disks for each treatment, weighing 1 g approximately, were individually placed in an 80-mL sterile filter bag (BagPage®, Interscience BagSystem, Saint Nom, France) and mixed with 9 mL of buffered peptone water (BPW, Biokar Diagnostics). They were smashed in a paddle blender (Minimix® 100, Interscience, France) for 120 s at 6 strokes s^{-1}. Aliquots were serially diluted in SP and 20 µL were plated by spot plating technique in duplicate plates of selective media. *L. monocytogenes* was plated in Palcam agar and *S. enterica* in XLD agar. Plates were incubated at 37±1 ºC for 48±2

h and at 37 ± 1 ºC for 24±1 h, for *L. monocytogenes* and *S. enterica*, respectively. Detection limit was 250 CFU g^{-1}. Results were transformed to log CFU g^{-1} and expressed as reductions in population growth, calculated as described in (Equation 1).

$$\text{Reduction (log units)}_d = (\log N_d/N_0) \qquad \text{Eq. 1}$$

Where N_0 is the mean of the population of untreated discs (as a population reference), and N_d is the population of each treatment at sampling date (d) (CFU g^{-1}).

2.3. Experimental design

Four sets of experiments were performed (Supplementary material, S1). The first trial consisted of the screening of the *in vivo* antimicrobial activity of ferulic acid (FA) against three *L. monocytogenes* and four *S. enterica* strains that were individually inoculated on apple and melon flesh disks. In the second, two methods for FA application (dipping or spraying) were compared against inoculated *L. monocytogenes* or *S. enterica* strains on apple and melon disks. In the third experiment, the minimum FA concentration against inoculated *L. monocytogenes* on apple and melon during storage was determined. Samples in trials involving pathogens were stored at 10 ºC for 7 d, in order to analyse the effect in a worse-case scenario of abusive storage temperature. Finally, the fourth set involved the evaluation of the fruit quality of uninoculated fresh-cut pieces treated with FA during storage at 4 ºC, mimicking the commercial conditions.

2.3.1. Effect of FA at different concentrations against pathogenic strains on fresh-cut apple and melon

Fruit discs were prepared and inoculated as described in section 2.2.2. Once dried, ten fruit disks per treatment were immersed in 500 mL of sterile distilled water containing FA in three different concentrations, low, medium, and high (FA-L, FA-M, FA-H) (Table 1), according to previous results at *in vitro* conditions (Nicolau-Lapeña, 2021). Another treatment, consisting of distilled water without FA, was added as control (CT). Ten discs were also left untreated, for population reference. After treatment, fruit discs were dried in a biosafety level 2 cabinet during 1 h. Microbial determinations were done as explained in section 2.2.3. immediately after the

164 treatments (D0) and after 7 d (D7) of storage at 10 ºC, being the disks stored in individual 15 mL-
165 glass tubes with a cap.

166 **2.3.2. Selection of application method: immersion or spray**

167 Preparation of apple and melon disks and culture of pathogenic strains is described in section
168 2.2.2. Two bacterial cocktails were prepared, one of *S. enterica* strains and another one of *L.*
169 *monocytogenes* strains, by mixing the 5 mL of the resuspended pellet of the cultured strains,
170 respectively. Inoculation of apple and melon disks with the respective cocktails was performed
171 as described in section 2.2.2.

172 Two application methods were studied: immersion and spray. For spray treatments, disks were
173 distributed in a lab rack, and sprayed with an airbrush model Hobby Air 707523 (Werther
174 International, Reggio Emilia, Italy) over one surface for 2 s each. Afterwards, discs were turned
175 over and were sprayed again. Three concentrations of FA were selected: 2.5, 5.0 and 7.0 g L^{-1}.
176 Also, a water application with no FA was added as a treatment control (CT), resulting in 8
177 different treatments, as a combination of immersion (I) or spray (S) and each of the FA
178 concentrations (CT, FA-2.5, FA-5.0, and FA-7.5). In addition, inoculated and untreated batches
179 of discs were included in the experiment and were the reference to compare reductions of
180 population. Results were expressed as described in Equation 1. Sampling dates were established
181 for D0 and D7. Populations were determined as explained in section 2.2.3.

182 Moreover, in the immersion treatment, a sample of water and FA wash water was analyzed after
183 treatment for pathogenic bacterial count, in order to check any bactericidal effect on wash water.
184 For this, duplicate 1 mL sample of water was mixed with 9 mL Dey-Engley neutralizing medium,
185 and serial dilutions were plated and incubated in duplicate on XLD or Palcam, for 24 or 48 h,
186 respectively, for *S. enterica* and *L. monocytogenes*. Results were expressed as log CFU mL^{-1}, and
187 detection limit was 50 CFU mL^{-1}. When counts were below the detection limit (<50 CFU mL^{-1}),
188 absence or presence of both pathogens in wash-water was determined by incubating the Dey-
189 Engley tubes at 37 ºC for 24 h. When presence was confirmed, a value corresponding to ½
190 detection limit was given for calculations.

2.3.3. Decreasing FA concentration against *L. monocytogenes*

The possibility to decrease FA concentration by maintaining the same antimicrobial efficacy was evaluated. For this, three concentrations were compared to FA-2.5: 1.0, 1.5, and 2.0 g L^{-1} FA (FA-1.0, FA-1.5, and FA-2.0) (n=3). Based on previous results, only *L. monocytogenes* cocktail on apple and melon was studied in this trial. Preparation of *L. monocytogenes* cocktail is described in section 2.2.1., and preparation of fruit disks, inoculation, and sampling times and procedure is described in sections 2.2.2 and 2.2.3. FA was applied by immersion, due to results obtained in previous experiments.

2.3.4. Effect of FA application on the quality of fresh-cut apple and melon

Non-inoculated fresh-cut fruit was used to evaluate commercial quality and shelf life of the fresh-cut apple and melon samples. Fresh-cut fruit (approximately 2.5 kg) was obtained from different fruits, which were previously surface disinfected in a 200 mg L^{-1} chlorine solution (pH adjusted to 6.5 using citric acid 2 M) for 2 min, and rinsed with tap water for 2 min. Then, apples were peeled and 10 wedges of approximately 1 cm width were obtained per fruit with a 10-blaze apple slicer and corer. For melon, pieces of approximately 4 × 3 × 2 cm without peel were cut with a knife. Fruit pieces were immersed in treatment solutions immediately after cutting. Pieces were randomly mixed and subjected to different treatments as follows.

FA treatments for apple and melon consisted of application of 2.5 g L^{-1} solution (FA-2.5) in a proportion 1:3 (fruit:solution) for 2 min. To study the antioxidant effect of FA, the commercial antioxidant NatureSeal® was the control treatment for fresh-cut apple (NS). Apple slices were immersed in NatureSeal® at 4 % (w:v) for 2 min, following provider instructions. Tap water was the control treatment for fresh-cut melon (W), in which was immersed for 2 min. Fruit pieces were let dry over a filter paper at room temperature for 1 h until packaging.

Fresh-cut apple and melon were stored in 500 mL clamshell plastic containers. Three clamshells, containing approximately 130 g apple or 200 g melon were prepared for each condition. Each container was considered a repetition. They were stored at 4±1 ºC until sampling date. The day

of the treatment was the first sampling date (D0). Apple was analyzed at days 5, 8, and 12 after treatment (D5, D8, D12), and melon at days 3, 5, and 7 after treatment (D3, D5, D7). Each sampling date, determination of pH, titratable acidity, total soluble solids, color, firmness, total aerobic mesophilic microorganisms, and yeasts and molds was performed in triplicate samples (three clamshells). Also, an aliquot of the fruit pieces was frozen using liquid nitrogen, pulverized in a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain), and kept at – 80 ºC for further biochemical determinations (antioxidant capacity, total polyphenol content). An aliquot was freeze dried for ferulic acid determination.

For **pH, titratable acidity (TA)** and **total soluble solids (TSS)** determination, the juice of three fruit pieces from 3 different containers (n=3) was obtained by means of a blender. pH was determined by using an electrode in a pH-meter GLP22 (Crison Instruments SA, Barcelona, Spain). For TA determination, 10 mL of juice were diluted with 10 mL of distilled water and titrated with 0.1M NaOH until pH 8.2 was reached. Results were expressed as malic acid for apple and citric acid for melon, in mg L^{-1}. TSS expressed as % was measured at 20 °C with a refractometer (Atago Co. Ltd., Tokyo, Japan).

Firmness was measured by the maximum penetration force using the TA.XT Plus Connect texture analyzer (Stable Micro systems Ltd., Surrey, England) on 10 fruits from each of the three containers (n=3). The maximum force encountered using a cylindrical probe (4 mm) at a speed of 5 mm s^{-1} and a trigger force of 0.1 N was determined.

Color was measured on 3 points of 10 fruit pieces from each of the three containers (n=3) by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan), with a D65 illuminant and a 10° observer angle. Color was expressed as CIE L*, a*, and b* coordinates. These values were used to calculate the browning index (BI) for fresh-cut apples, using the equation proposed by (Pathare et al., 2013) (Equation 2) and the total color difference (TCD) for fresh-cut melon (Equation 3):

$$BI = 100 \times \left(\frac{X-0.31}{0.17}\right) \quad \text{Eq. 2.1} \qquad \text{where} \quad X = \frac{(a* + 1.75\,L*) \times a*}{(5.645L* + a* - 3.012b*)} \quad \text{Eq. 2.2}$$

243 $TCD = ((L^*_d - L^*_0)^2 + (a^*_d - a^*_0)^2 + (b^*_d - b^*_0)^2)^{0.5}$ Eq. 3

244 where d=value at sampling day and 0=initial value (value at D0).

245 **Overall acceptance** of the fruit pieces was determined by sensory evaluation by habitual
246 consumers of this kind of products (n=20). Fruit pieces with the different treatments were
247 presented with a random codification and consumers evaluated acceptance in a 9-point hedonic
248 scale.

249 To determine **total aerobic mesophilic microorganisms (TAM)** and **yeasts and molds (Y&M)**
250 counts, 10 ± 1 g of three different fruit pieces per triplicate (n=3), to assure heterogeneity, were
251 mixed with 90 mL PS in a sterile filter bag (BagPage®, Interscience BagSystem, Saint Nom,
252 France) and homogenised using a paddle blender (Minimix®, Interscience, France) for 120 s at
253 12 strokes s-1. Aliquots were diluted in SP and plated in duplicate plates. For TAM, samples were
254 plated in PCA and incubated at 30±1 ºC for 3 d. For Y&M, samples were plated in DRBC and
255 incubated at 25±1 ºC for 5 d. Detection limit was 50 CFU g^{-1}.
256 Ferric reducing antioxidant power (FRAP) and DPPH· scavenging radical tests were used to
257 determine the **antioxidant capacity** (n=3). **Total phenolic content** (TPC) was determined by
258 Folin -Ciocalteau method (n=3). For the extraction, 3.0±0.1 g were mixed with 10 mL of methanol
259 70% (v/v) and homogenized in a vortex. After stirring at 4 °C for 20 min, the samples were
260 centrifuged by means of a Sigma-3-18 KS centrifuge (Sigma Laborzentrifugen GmbH, Osterode
261 am Harz, Germany) at 13 500 × *g* for 20 min at 4 °C. Supernatant was then filtered and marked
262 to 12.5 mL with methanol 70 %. FRAP, DPPH· and TPC determinations were performed as
263 described in Nicolau-Lapeña et al. (2019). Results of antioxidant capacity by FRAP and DPPH·
264 methods were expressed as ascorbic acid equivalents in g kg^{-1}. Results of TPC were expressed as
265 gallic acid equivalents in g kg^{-1}.

266 **Evaluation of FA in the samples**. To determine the concentration of FA that remained in fruit
267 disks after the immersion in the solutions, an extraction of the phenolic content was carried out
268 by mixing 3.0 ± 0.1 g of the frozen dried sample with 10 mL of a methanol solution 70 % (v:v).
269 After agitation for 20 min, samples were centrifuged (14000 × *g* for 10 min) and the supernatant

was further lyophilized. It was then resuspended in water, methanol and formic acid (1:98:1 v:v:v) and determined by UPLC-MS, using Acquity UPLC-Xevo TQS (Waters). UPLC was performed using Acquity UPLC ® HSS T3 1.8 µm, 150 x 2.1 mm column, injecting 5 µL of sample at 10 ºC, in a isotherm column at 40 ºC, with two mobile phases: (A) water, methanol and formic acid (98:1:1 v:v:v) and (B) methanol and formic acid (99:1.5 v:v) at 0.3 mL min^{-1} in a gradient as follows: from 0 to 0.51 min 80 % A and 20 % B, from 0.51 to 5.00 min, 20 % A and 80 % B, from 5.01 to 7.50, flow was increased to 0.4 mL min and mobile phases were 1 % A and 99 % B. Finally, back at initial conditions to 10.00 min. Mass spectrometry was done with an ESI with negative ion mode, 2 kV capillarity, source and desolvation temperatures, 120 and 450 ºC, respectively, desolvation gas flow was 1000 L h^{-1}, and collision gas flow was 0.15 mL min^{-1}. Multiple reaction monitoring of ferulic acid in channels 192.83 > 134.20 and 192.83 > 177.97, where collision energy was 15 eV and Cone was 30 V. Results were expressed as g kg^{-1} (dry weight basis), and detection limit was 0.026 mg kg^{-1}.

2.4. Statistical analysis

All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. All statistical analyses were carried out using JMP 13 (SAS Institute Inc., Cary, USA).

3. Results

3.1. Setting up experimental conditions

In the evaluation of the antimicrobial effect of FA, solutions of different FA concentrations were used (sections 2.3.1. (Table 1), 2.3.2., 2.3.3.). The pH values of FA solutions did not differ significantly between concentrations and ranged from 3.9±0.1 to 3.7±0.1, from the lowest to the highest concentration (1.0 to 15.0 g L^{-1}). Quality main parameters (pH, TSS and TA) of the fruits used in these trials are shown in Table 2.

295 Prior to the treatments, apple discs were inoculated with *S. enterica* or *L. monocytogenes*
296 concentrates. In experiments performed with individual strains, populations of untreated discs at
297 D0 ranged from 5.5±0.2 to 6.5±0.1 in apple and averaged 6.3±0.2 log CFU g^{-1} in melon (Table
298 3). In general, *L. monocytogenes* strains reached higher populations than *S. enterica* after storage
299 (7 d at 10 ºC). Only *S.* Agona decreased by 1 log on apple and increased by 1.7 log on melon after
300 7 d of storage. In fact, differences in growth for each strain were observed between fruit matrices:
301 higher growth was observed in melon disks when compared to apple disks. For experiments using
302 a cocktail of *S. enterica* or a cocktail of *L. monocytogenes*, populations of such pathogens in
303 untreated apple and melon were 6.3±0.1 and 6.1±0.1 log CFU g^{-1}, respectively. For experiments
304 involving only *L. monocytogenes*, initial populations on apple and melon disks were 5.9±0.3 and
305 6.2±0.1 log CFU g^{-1}, respectively (Table 4).

306 **3.2. Bactericidal effect of FA against *S. enterica* and *L. monocytogenes* in fresh-cut apple**
307 **and melon.**

308 Bactericidal effect of FA was evaluated immediately after the treatments and the drying time, by
309 comparing populations of the untreated control with those of the treatments. For instance, in the
310 first experiment using individual strains, the values of pathogen population at D0 and D7 in
311 untreated samples (Table 3) were used as a population control to calculate the log reductions
312 (Eq.1) for each treatment shown in Figure 1. Immediately after the treatments, populations were
313 reduced by 0.4±0.2 or 0.3±0.1 log units in apple or melon disks, respectively, regardless of the
314 FA concentration (data not shown). These reductions were considered negligible for an
315 antimicrobial treatment, implying no immediate bactericidal effect of FA. Similar results were
316 obtained in further experiments evaluating the bactericidal effect of FA on samples inoculated
317 with the microbial cocktails. Overall, no remarkable differences were observed between
318 application methods or FA concentrations, with reductions of each studied microorganism and
319 fruit < 0.5 log units.

320 **3.3. Effect of FA in controlling *S. enterica* and *L. monocytogenes* growth on fresh-cut apple**
321 **and melon during storage.**

The effect of FA at different concentrations and different application methods was evaluated after 7 d of storage at 10 ºC. In the first experiment using individual strains (Figure 1), pathogen reductions in FA treated apple and melon were significantly higher than they were in fruit disks washed with water (control treatment, CT). This fact implied that, while pathogens in CT samples grew under storage conditions similarly to untreated sample, pathogens in FA samples did not grow that much, or even decreased when compared to D0. Regarding *S. enterica* strains, *S*. Agona (Figure 1A) showed a different behaviour depending on the fruit matrix. As indicated above (Table 3), values decreased in untreated apple discs stored at 10 ºC, and the addition of FA did not enhance this decreasing effect. In melon, in contrast, this strain grew in the untreated sample, but decreased 2.0±0.1 log in FA treated samples. *S*. Montevideo (Figure 1B) in apple was reduced by 3.6±1.4 log by all FA treatments. In melon, a significant difference between FA-L and FA-H was observed, with reductions of 0.8±0.4 and 1.9±0.3 log, respectively. *S.* Gaminara (Figure 1C) strain was not affected by FA immersion. Differences between control treatment (CT) and FA treatments were not significant, in either apple or melon. *S.* Typhimurium (Figure 1D) population in samples treated with FA was reduced 1.5±0.2 and 2.3±0.1 log in apple, and between 2.2±0.2 and 2.7±0.1 log in melon, when compared to the untreated reference. Concerning the effect of FA against *L. monocytogenes*, higher reductions than those obtained in *S. enterica* were observed, even higher concentrations were used for *S. enterica* (5.0 to 15.0 g L^{-1} FA) in comparison to those used for *L. monocytogenes* (2.5 to 10.0 g L^{-1}). Therefore, *L. monocytogenes* was more susceptible than *S. enterica* to FA. In comparison to the untreated control, *L. monocytogenes* 1/2, 4b, and 1/2a growth at the end of storage (7 d, 10 ºC) was reduced by 4.0±0.2 log in apple, and ranged from 2.0±0.4 to 4.7±0.2, from 3.2±0.2 to 3.6±0.6, and from 3.9±0.1 to 4.1±0.2 log in melon, respectively.

Different application methods may have further different effects, as they can influence the amount of solution absorbed, the surface covered, and the concentration of active compounds to use. For this experiment, *S. enterica*, tested concentrations were decreased, because no difference was observed between FA-L and FA-H in the previous experiment. Therefore, a lower product

concentration was studied, expecting the same effect. Moreover, concentrations > 7.5 g L^{-1} clogged the nuzzle. Regarding *S. enterica* cocktail (Figure 2A), the reduction of population in apple discs at the end of storage (7 d, 10 ºC) was lower than 1 log unit regardless of the method of application and tested FA concentration. In melon, the application of FA by immersion (I-FA) caused a slightly higher reduction than application by spray (S-FA). In contrast, reductions after storage (7 d, 10 ºC) caused by immersion application method against *L. monocytogenes* cocktail were significantly higher than those caused by spray application for each studied fruit (Figure 2B). Reductions were 2.1- and 2.8-fold higher in I-FA compared to S-FA, in apple and in melon, respectively. No differences were observed between FA-2.5, FA-5.0, or FA-7.5, in any of the cases. Due to its higher efficacy against *L. monocytogenes*, application of FA by immersion was selected for further experiments.

As it has been described before, *L. monocytogenes* was effectively controlled by concentrations of FA above 2.5 g L^{-1}, while higher concentrations of FA were needed to reduce *S. enterica*. In this trial, the use of lower FA concentrations against *L. monocytogenes* was tested. At the end of storage (D7) at 10 ºC, *L. monocytogenes* reductions in apple disks ranged from 3.7±0.1 to 4.1 ± 0.1 log units, compared to the untreated reference. In contrast, in melon disks, *L. monocytogenes* reduction values were positively correlated to the FA concentration applied, and significantly affected its efficacy, with reduction values ranging between 1.0±0.1 and 3.9±0.1 log.

3.4. Effect of FA in the control microorganisms in the wash water

The remaining microorganisms in the wash water (*S. enterica* and *L. monocytogenes*) were evaluated after the treatments to investigate whether FA could act also as a control to maintain safety of the washing-water and to prevent cross-contaminations. However, the counts in the FA solution or water after the fruit immersions revealed the presence of 4.9 ± 0.1 or 4.7 ± 0.3 log CFU mL^{-1} of *S. enterica* or *L. monocytogenes*. I-FA-2.5, I-FA-5.0 and I-FA-7.5 solutions contained the same concentration of microorganisms after treatments, confirming no bactericidal effect caused by FA at these concentrations (Data not shown).

3.5. Impact of FA in the quality of fresh-cut apple and melon

The effect that FA selected dose (2.5 g L^{-1}) had in the quality of fresh-cut apple and melon was determined in non-inoculated samples, and fruit pieces were stored at 4 ºC to mimic commercial conditions, during 12 or 7 d, for apple or melon, respectively. The determination of the FA by HPLC-MS in the fresh-cut samples revealed that apple pieces contained 0.25±0.04 g kg^{-1} (dry weight basis), and melon pieces contained 1.22±0.07 g kg^{-1} (dry weight basis).

No significant differences were observed in quality parameters (pH, TSS and TA) of apple or melon during storage, regardless of the treatment. In apple, values for these parameters were 4.3 ± 0.1, 11.6±0.6 %, and 3.1±0.3 g L^{-1}, respectively. In melon, these values were 5.9±0.1, 11.4±0.2 % , and 1.6±0.1 g L^{-1}, respectively.

Firmness of fresh-cut apples after the treatments was 13.84±0.24 N, and 10.09±0.75 N, for Natureseal® treatment (NS) and 2.5 g L^{-1} FA (FA-2.5), respectively (Table 5). During storage, firmness of NS samples increased and firmness of FA-2.5 significantly decreased, achieving values up to 18.14±1.36 and 6.85±0.17 N, respectively at day 12 of storage. In fresh-cut melon, both water control (W) and 2.5 g L^{-1} FA (FA-2.5) samples had the same firmness values immediately after the immersion in the treatment solutions, which averaged 5.76 ± 0.09 N. However, firmness values significantly decreased up to 4.88 ± 0.02 N after 7 d of storage.

Regarding color, the initial L*, a*, and b* coordinates of apple wedges were 79.8±0.5, 1.5±0.3, and 19.4±0.7, respectively (Supplementary figure 2, S2). The changes observed led to a reduction in luminosity and an increase in reddish color, which can be expressed by browning index (BI) (Figure 3A). In NS samples, BI value was maintained during storage, but BI in FA-2.5 treated fresh-cut apples increased from 4.0±0.3 to 10.7±1.0. In melon pieces, initial L*, a*, and b* values were 69.8±0.3, 1.1±0.1, and 6.7±0.1, respectively (Supplementary figure 2, S2). Overall, although there were some variations during storage, these values did not show significant differences between W control and FA-2.5 treatment. At D7, TCD of samples averaged 1.4±0.2 for both treatments (Figure 3B).

401 The sensory evaluation revealed that apple wedges treated with FA-2.5 had lower acceptance than
402 those with NS (Table 6). Comments revealed that consumers had perceived in those samples an
403 acid aftertaste and a softer texture when compared with NS control. Contrarily, acceptance of FA-
404 2.5 treated melon wedges was not different from that of W control (Table 6).

405 Control apple wedges treated with NS treatment showed significantly higher antioxidant values,
406 both in DPPH· and in FRAP methods, than samples treated with FA-2.5 did (Table 7). During
407 storage, DPPH· and FRAP significantly decreased, although in FA-2.5 such decrease was
408 delayed. At the end of storage, antioxidant values of NS and FA-2.5 samples decreased by 37.3
409 and 25.7 %, respectively. In melon, the addition of FA-2.5 to samples increased by 1.6-, 5.7-, and
410 3.2-fold their FRAP, DPPH· antioxidant capacities, and TPC values were also higher when
411 compared to W samples (water control). A decrease in DPPH· and TPC values of fresh-cut melon
412 was observed during storage, achieving final values of 12.48±0.42 · 10^{-2} g kg^{-1} and 6.61±2.08 ·
413 10^{-2} g kg^{-1}, respectively.

414 Initial population of TAM in fresh-cut apples was 2.4±0.4 log CFU g^{-1} (Figure 4A). Immediately
415 after NS and FA-2.5 treatments, TAM decreased to 2.1±0.1 and 1.8±0.5 log, respectively. During
416 the 8 d of storage, counts increased similarly in both samples. In the case of fresh-cut apple, the
417 treatment with FA showed a bacteriostatic effect, as populations did not significantly increase
418 during this time. After 12 days of storage (D12), TAM count in FA-2.5 samples was maintained
419 at 3.1±0.1 log CFU g^{-1}, while counts in NS samples achieved 4.5±0.3 log CFU g^{-1}. In melon
420 (Figure 4B), contrarily, although the initial TAM counts were 2.6±0.1 log CFU g^{-1}, and after
421 immersion of wedges in water control (W) or FA-2.5 solutions, counts decreased to 1.5±0.2 and
422 1.4±0.1 log, respectively, growth was not controlled by any of the treatments during storage. At
423 D7, TAM counts in fresh-cut melon were 5.5±0.3 log CFU g^{-1}, for both W or FA-2.5 treatments.

424 Initial Y&M population in apple wedges was 1.6±0.4 log CFU g^{-1}, and it did not significantly
425 decrease after immersion in NS or FA-2.5 solutions (Figure 4C). Y&M counts increased during
426 storage similarly for both treatments, and after 12 d at 4 ºC, it was 3.3±0.5 and 3.0±0.2 log, for
427 NS and FA-2.5, respectively. Contrarily, Y&M populations in fresh-cut melon remained stable

428 for the first 5 d of storage at 1.4±0.1 log CFU g^{-1}(Figure 4D). At the end of storage (7 d),
429 population in water control (W) samples grew up to 2.4±0.1 log CFU g^{-1}, while it remained in
430 FA-2.5 samples.

431

4. Discussion

The antimicrobial effect of ferulic acid was studied in *L. monocytogenes* and *S. enterica* inoculated in fresh-cut apple and melon. Results revealed no bactericide but bacteriostatic effect during the 7 d of storage at 10 ºC for the 7 strains used. For this, FA could be suggested as a solution to prevent pathogenic bacterial growth in fresh-cut products. According to the European regulations on microbiological safety (Reg. EC 2073/2005 and subsequent modifications), the criteria for fresh-cut fruit are the following: *Salmonella* spp. must not be detected in 25 g (5 samples) during the products' shelf-life and *Listeria monocytogenes* should not be detected in 25 g (5 samples) at the end of the production chain and should be maintained under 10^2 CFU g^{-1}. The application of FA would help in meeting the shelf-life criteria for fresh-cut fruits, as if selected pathogens are present but not detected at the end of production chain, its application can maintain such counts below the regulation limit during the storage of the product.

In general, all strains of each microorganism were affected in the same way by showing reductions around 2 to 4 log units. FA has already been reported to have antimicrobial effects against *L. monocytogenes* (Borges et al., 2013; Pernin et al., 2019b). In fact, in previous studies carried out by our investigation group, it was found that *L. monocytogenes* was more affected by FA than other tested strains such as *Bacillus cereus*, *Escherichia coli* or *Salmonella enterica* when tested *in vitro*. In this paper, the concentrations selected for the first trial were at least 2 times higher than the MIC found in our previous studies (ranging from 1.7 to 3.3 g L^{-1}), because it has been observed that the concentration remaining in fruit tends to be lower than the concentration at which it is immersed. In fact, when fresh-cut apple and melon were immersed in a solution containing 2.5 g L^{-1} FA, the remaining content was 0.25±0.04 and 1.22±0.07 g kg^{-1} (dry weight basis), respectively. In line with the results obtained in this paper, Takahashi et al. (2013) reported no remarkable effect of FA on Gram negative bacteria, including *Salmonella* spp. The FA action mode combines two mechanisms; the acidic and the lipophilic mechanisms. The acidification of the cell cytoplasm, together with a K^+ ions efflux caused by the dissociation of the acid leads to an eventual death of the bacterial cells. Also the transport of the substances across the membrane

459 is inhibited by a disturbance in the Van der Waals forces, occurring when the acid is intercalated
460 in the phospholipid layers of the membrane (Pernin et al., 2019a).

461 In this study and based on previous results of the research group, three different concentrations
462 of FA were tested for each strain. Except for *S.* Typhimurium in fresh-cut apples, and *S.*
463 Montevideo and *L. monocytogenes* 1/2a in fresh-cut melon, the antimicrobial effect observed was
464 not concentration-dependent at the tested doses. Even though previous *in vitro* studies carried out
465 in our lab indicated that the concentration chosen for FA-M treatment was the MIC for each strain,
466 *in vivo* trials are needed to consider the different variables, including the food characteristics,
467 namely pH, natural antimicrobials, roughness of surface and adhesion capability of the cells to it,
468 and extrinsic factors such as storage temperature. In the present study, we observed that FA had
469 a bacteriostatic effect, not bactericidal.

470 The decrease in *S. enterica* or *L. monocytogenes* differed depending on the fruit studied. The
471 difference in the behavior of these bacteria under the same concentrations in apple or melon could
472 be related to the intrinsic properties of the sample, such as pH, acidity or the type of the
473 characteristic acids. Apple and melon pH values were 4.6 ± 0.3 and 5.7 ± 0.3 (Table 2) and malic
474 and citric are the predominant acids, respectively. The higher pH and lower acidity of the melon
475 may facilitate the growth of the microorganisms when compared to apples. Therefore, pH is acting
476 as a hurdle preventing growth of *L. monocytogenes* by itself in those samples, which makes lower
477 reduction values.

478 The concentrations of FA used against *L. monocytogenes* were reduced from 2.5 to 1.0 g L^{-1} as it
479 was observed that concentrations of 2.5 showed a higher antimicrobial effect against *L.*
480 *monocytogenes* than against *S. enterica*. That reduction in FA concentration was accompanied
481 with a reduction in its efficacy in apple, but not in melon. When FA was applied at concentrations
482 higher than 2.5 g L^{-1}, the antibacterial effect was similar for all of them, independently of the
483 concentration tested. One possible explanation is that independently of the concentration in the
484 washing solution, the FA that remained on the surface of the apple was the same, because there
485 could be a maximum surface / FA attachment ratio that was already reached at 2.5 g L^{-1}. This

attachment ratio could depend on the porosity of the matrix. In fact, the difference in applying the same concentration of FA to different fruit matrices (apple and melon) was patent when determining the remaining FA in their surfaces: it was 6 times higher in melon than it was in apple (1.22±0.07 g kg^{-1} and 0.25±0.04 g kg^{-1} (dry weight basis), respectively). FA has also been tested for *L. monocytogenes* growth inhibition in food matrices other than fruit. For example, Takahashi et al. (2013) added FA at 2 or 4 mg g^{-1} of cheese or salmon, respectively, and observed that inoculated *L. monocytogenes* did not grow as much as the non-FA control did (2 or 3 log units in FA-treated cheese or salmon, compared to 5 logs in non-treated samples, after the end of the storage). This highlights the need to evaluate the effect against pathogens both *in vitro* and *in vivo*, as the target matrix characteristics may interfere or interact with the antimicrobial agent or the pathogen in several ways. In fact, Belgacem et al. (2020) also found differences between matrices (apple, melon and pear) when investigating the effect of a pomegranate peel extract (PGE) on the growth of *L. monocytogenes*.

Moreover, two different application methods (immersion and spray) were evaluated, because depending on the properties of the solution and the product characteristics, they may have different performances (Zhong et al., 2014). Other studies did not show differences in the effect of antimicrobial essential oils on lettuce between these two application methods in mesophilic, psychrotrophic, and coliform bacteria (Ponce et al., 2011). In the present study, however, the immersion application method was selected over the spraying, because it was more effective in inhibiting growth of pathogens, probably because of a greater impregnation of the product. Also, 2.5 g L^{-1} of FA proved to be effective against *L. monocytogenes* but also in *S. enterica*, so to assure the efficacy in both species, this concentration was selected to continue with the following experiments.

Finally, FA preserved the quality parameters pH, TSS or TA of the studied fresh-cut products, which did not vary, and were in accordance with those found in the literature (Iglesias and Alegre, 2006; Kolayli et al., 2010). Regarding textural quality, the application of NS in apple resulted in a decrease in firmness when compared to the control. As also observed by Rössle et al. (2009),

513 Natureseal® reduced firmness loss in consequence of cross-linking cell wall and middle-lamella
514 pectin (Rössle et al., 2009). A decrease in firmness of apple wedges was observed during storage
515 in FA-treated samples. On the contrary, the firmness of samples in the control treatment (NS) was
516 maintained or even increased and was significantly higher than FA treated fresh-cut apple. In
517 melon, the treatment FA-2.5 did not maintain firmness, which decreased with time comparably
518 to the W control. Texture loss could probably be attributed to to enzyme activities, such as
519 galactosidase, endo- polygalacturonase, and/or exo-polygalacturonase, which solubilize pectin in
520 cell walls of melon pieces (Aguayo et al., 2004).

521 Color can suggest freshness and flavor qualities to consumers (Barrett et al., 2010). Browning is
522 a product alteration easily detected by consumers, which leads to product rejection (Jaeger et al.,
523 2018). BI was used as a pointer of color quality in fresh-cut apples, which are highly affected by
524 these reactions (Lunadei et al., 2010). NS was selected as a commercial antioxidant treatment to
525 use in the fresh-cut apple processing industry. FA is considered to be an antioxidant and its
526 polyphenol oxidase activity (PPO) inhibition capacity has been associated with it. It can prevent
527 the binding between substrate and enzyme by occupying the latter's active place (Shannon and
528 Pratt, 1967). In this study, FA did not behave as an anti-browning agent as NS treatment did.
529 Previous work of our investigation group (Nicolau-Lapeña et al., 2021) reported that 2.5 g L^{-1}
530 inhibited 21.2 ± 1.9 % the apple PPO activity. Maybe, regardless of its reported PPO inhibitory
531 activity at *in vitro* conditions, more concentration is needed to increase its visible anti-browning
532 effect in apples. We have to take into account that fruit was stored under air conditions (not in a
533 modified atmosphere) and oxygen could facilitate browning. For this, further investigations
534 would be needed, including the use of modified atmospheres or the combination with FA for
535 pathogenic control and NS for color preservation. In melon, the TCD values averaging 1.4
536 indicate that color was well maintained during storage (Mokrzycki and Tatol, 2011).

537 The antioxidant capacity of a fruit can increase its stability during storage and prevent detrimental
538 changes, including variations in color (Hassimotto et al., 2005). Apples treated with NS had
539 higher antioxidant capacity than they had with FA-2.5. TPC values were also significantly higher

in NS samples than they were in FA-2.5 samples. As NS does not contain phenolic compounds, the higher TPC values could be attributed to an overestimation of TPC by interference caused by ascorbate, which is included in the composition of Natureseal®. Ascorbic acid is a reducing compound (non-phenolic antioxidant), which also reduces the Folin-Ciocalteau reagent to form a blue color in alkaline pH (Lester et al., 2012). On the other hand, in melon, FA-2.5 samples showed higher antioxidant values when compared to W control. In fact, FA has already been reported to be a powerful antioxidant (Zduńska et al., 2018). Moreover, FA helped to maintain the antioxidant capacity of melon during storage and the TPC content remained constant.

The effect of FA on native microbiota of apple and melon was also studied. Immediately after treatments, populations of TAM slightly decreased, possibly because of the soaking in agitated water. However, only FA-2.5 treatment in apple was able to control TAM populations after 12 d of storage (4 ºC). Regarding Y&M, FA was not effective in decreasing or controlling populations in apples or melons. Moreover, the reductions in natural microbiota were lower than they were in the inoculated pathogens. Even some authors have reported that FA at concentrations higher than 250 mg L^{-1} would have antimicrobial effect against *Saccharomyces cerevisiae* (Baranowski et al., 1980), there is not much literature on how FA may affect growth of yeasts and molds. Thus, more studies on effective concentrations and action modes should be carried out in the future. Although there is not a legislation determining the non-pathogenic native microbiota in fresh-cut products, the final concentrations of TAM reached 5 log units per gram, from which, 2 to 3 log units were Y&M. The high levels of microorganisms could alter the food's appearance, odor, texture, or taste, because of their biochemical activity as they grow in the food, that can include carbohydrate degradation into simpler sugars, organic acid oxidation or sugar fermentation (Sperber, 2009).

Conclusions

In this paper, the application of ferulic acid (FA) in fresh-cut apples and melons was evaluated. Immersion method was selected over spray application for FA, as it proved to have higher efficacy. Although no bactericidal effect after washing was found against the studied pathogenic microorganisms (*L. monocytogenes* and *S. enterica*), FA at 2.5 g L^{-1} highly prevented growth of *L. monocytogenes* on fresh-cut apple and melon during storage at 10 ºC for 7 d, without affecting the quality evaluated in fresh-cut apple and melon stored at 4 ºC for 12 and 7 d, respectively. Some effect was found against *S. enterica*, but populations in fresh-cut fruit remained relatively high after storage at 10 ºC for 7 d. Moreover, the reported health impact that FA may exert, including anti-inflammatory, anti-thrombosis and anti-cancer activities, could contribute to enhancing nutritional and functional properties of fresh-cut fruit, adding value to these products for the consumers' benefit. An optimisation of the formula would be needed in order to minimize aftertastes detected in apple. Moreover, quality maintenance during storage should be improved, maybe by combining FA with another preservative as NS.

Overall, FA effect in delaying the growth of pathogenic microorganisms, *L. monocytogenes* and *S. enterica*, would present this substance as a potential ingredient or additive to be used in fresh-cut products, in order to offer consumers safe and quality products. In a possible real application, the use of FA should be accompanied by the disinfection step, as no disinfection effect has been demonstrated in the studied conditions. However, legislation, scale up, and other pathogenic strains or fruit matrices should be also evaluated when developing commercial products using this compound.

583 **Acknowledgements**

584 The authors would like to thank the AGRIMAX project for funding the research. AGRIMAX is
585 funded by the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020
586 Research and Innovation Programme under grant agreement No. 720719. This work was also
587 supported by the CERCA Programme of 'Generalitat de Catalunya'.
588 I. Nicolau-Lapeña is in receipt of a predoctoral grant awarded by the 'Ministerio Español de
589 Economía, Industria y Competitividad' (grant number BES-2017-079779). Dr. I. Aguiló-Aguayo
590 thanks the National Programme for the Promotion of Talent and Its Employability of the
591 'Ministerio de Economía, Industria y Competitividad' of the Spanish Government and the
592 European Social Fund for her Postdoctoral Senior Grant 'Ramon y Cajal' (RYC-2016-2019 949).
593 Authors also thank Sara Acosta and Silvia Villaró for their technical support
594
595
596 **Conflicts of interest**

597 The authors declare no conflict of interests.

598 **References**

599 Abadias, M., Alegre, I., Oliveira, M., Altisent, R., Viñas, I., 2012. Growth potential of
600 *Escherichia coli* O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and
601 escarole) stored under different conditions. Food Control. 21, 37–44.

602 Abadias, M., Alegre, I., Usall, J., Torres, R., Viñas, I., 2011. Evaluation of alternative sanitizers
603 to chlorine disinfection for reducing foodborne pathogens in fresh-cut apple. Postharvest Biol.
604 Technol. 59, 289–297. https://doi.org/10.1016/j.postharvbio.2010.09.014

605 Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2008. Microbiological quality of fresh,
606 minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food
607 Microbiol. 123, 121–129. https://doi.org/10.1016/j.ijfoodmicro.2007.12.013

608 Aguayo, E., Escalona, V., Artés, F., 2004. Metabolic behavior and quality changes of whole and
609 fresh processed melon. J. Food Sci. 69, 148–155.

610 Baranowski, J.D., Davidson, P.M., Nagel, C.W., Branen, A.L., 1980. Inhibition of
611 *Saccharomyces cerevisiae* by naturally occuring hydroxycinnamates. J. Food Sci. 45, 592–594.
612 https://doi.org/10.1111/j.1365-2621.1980.tb04107.x

613 Barrett, D.M., Beaulieu, J.C., Shewfelt, R., 2010. Color, flavor, texture, and nutritional quality of
614 fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the
615 effects of processing. Crit. Rev. Food Sci. Nutr. 50, 369–389.
616 https://doi.org/10.1080/10408391003626322

617 Belgacem, I., Schena, L., Teixidó, N., Romeo, F., Ballistreri, G., Abadias, M., 2020. Effectiveness
618 of a pomegranate peel extract (PGE) in reducing *Listeria monocytogenes* in vitro and on fresh-cut
619 pear, apple and melon. Eur. Food Res. Technol. 246, 1765–1772. https://doi.org/10.1007/s00217-
620 020-03529-5

621 Borges, A., Ferreira, C., Saavedra, M.J., Simo, M., 2013. Antibacterial Activity and mode of
622 action of ferulic. Microb. Drug Resist. 10, 1–10. https://doi.org/10.1089/mdr.2012.0244

623 Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C.,
624 Troncoso, A.M., 2015. Reported foodborne outbreaks due to fresh produce in the United States
625 and European Union: Trends and causes. Foodborne Pathog. Dis. 12, 32–38.
626 https://doi.org/10.1089/fpd.2014.1821

627 Grau Rojas, A., Garner, E., Martín Belloso, O., 2010. The fresh-cut fruit and vegetables industry,
628 current situation and market trends, in: Martín Belloso, O., Soliva Fortunt, R. (Eds.), Advances
629 in Fresh-Cut Fruits and Vegetables Processing. Taylor and Francis Group, pp. 1–12.

630 Hassimotto, N., Genovese, M., Lajolo, F., 2005. Antioxidant activity of dietary fruits, vegetables,
631 and commercial frozen fruit pulps. J. Agric. Food Chem. 53, 2928–2935.

632 Iglesias, I., Alegre, S., 2006. The effect of anti-hail nets on fruit protection, radiation, temperature,
633 quality and profitability of 'Mondial Gala' apples. J. Appl. Hortic. 8, 91–100.

634 Jaeger, S.R., Machín, L., Aschemann-Witzel, J., Antúnez, L., Harker, F.R., Ares, G., 2018. Buy,
635 eat or discard? A case study with apples to explore fruit quality perception and food waste. Food
636 Qual. Prefer. 69, 10–20. https://doi.org/10.1016/j.foodqual.2018.05.004

637 Kolayli, S., Kara, M., Tezcan, F., Erim, F.B., Sahin, H., Ulusoy, E., Aliyazicioglu, R., 2010.
638 Comparative study of chemical and biochemical properties of different melon cultivars: Standard,
639 hybrid, and grafted melons. J. Agric. Food Chem. 58, 9764–9769.
640 https://doi.org/10.1021/jf102408y

641 Kumar, N., Pruthi, V., 2014. Potential applications of ferulic acid from natural sources.
642 Biotechnol. Reports 4, 86–93. https://doi.org/10.1016/j.btre.2014.09.002

643 Lester, G.E., Lewers, K.S., Medina, M.B., Saftner, R.A., 2012. Comparative analysis of
644 strawberry total phenolics via Fast Blue BB vs. Folin-Ciocalteu: Assay interference by ascorbic
645 acid. J. Food Compos. Anal. 27, 102–107. https://doi.org/10.1016/j.jfca.2012.05.003

646 Lunadei, L., Galleguillos, P., Diezma, B., Lleó, L., 2010. Evaluation of enzymatic browning in
647 fresh-cut apple slices applying a multispectral vision system. Conf. Agric. Eng. 2010 Towar.
648 Environ. Technol. Clermont-Ferrand, Fr. 6-8 Sept. 2010. Cemagref. 1–11.

649 Mattila, P., Kumpulainen, J., 2002. Determination of Free and Total Phenolic Acids in Plant-
650 Derived Foods by HPLC with Diode-Array Detection. J. Agric. Food Chem. 50, 3660–3667.
651 https://doi.org/Mattila, P., & Kumpulainen, J. (2002). Determination of Free and Total Phenolic
652 Acids in Plant-Derived Foods by HPLC with Diode-Array Detection. Journal of Agricultural and
653 Food Chemistry, 50(13), 3660–3667. doi:10.1021/jf020028p

654 Mokrzycki, W., Tatol, M., 2011. Color difference Delta E - A survey. Mach. Graph. Vis. 20, 383–
655 411. https://doi.org/1230-0535

656 Nicolau-Lapeña, I., Abadias, M., Bobo, G., Aguiló-Aguayo, I., Lafarga, T., Viñas, I., 2019.
657 Strawberry sanitization by peracetic acid washing and its effect on fruit quality. Food Microbiol.
658 83, 159–166. https://doi.org/10.1016/j.fm.2019.05.004

659 Pacheco-Ordaz, R., Wall-Medrano, A., Goñi, M.. M., Ramos-Clamont-Montfort, G., Ayala-
660 zavala, J.F., González-Aguilar, G.A., 2017. Effect of phenolic compounds on the growth of
661 selected probiotic and pathogenic bacteria. Lett. Appl. Microbiol. 12, 3218–3221.
662 https://doi.org/10.1111/ijlh.12426

663 Pathare, P.B., Opara, U.L., Al-Said, F.A.J., 2013. Colour measurement and analysis in fresh and
664 processed foods: A review. Food Bioprocess Technol. 6, 36–60. https://doi.org/10.1007/s11947-
665 012-0867-9

666 Pernin, A., Bosc, V., Maillard, M.N., Dubois-Brissonnet, F., 2019a. Ferulic acid and eugenol have
667 different abilities to maintain their inhibitory activity against Listeria monocytogenes in
668 emulsified systems. Front. Microbiol. 10, 1–10. https://doi.org/10.3389/fmicb.2019.00137

669 Pernin, A., Guillier, L., Dubois-brissonnet, F., 2019b. Inhibitory activity of phenolic acids against
670 *Listeria monocytogenes*: Deciphering the mechanisms of action using three di ff erent models. J.
671 Food Microbiol. 80, 18–24. https://doi.org/10.1016/j.fm.2018.12.010

672 Pinela, J., Ferreira, I.C.F.., 2015. Nonthermal physical technologies to decontaminate and extend
673 the shelf-life of fruits and vegetables: Trends aiming at quality and safety. Crit. Rviews Food Sci.
674 Nutr. 57, 2095–2111.

675 Ponce, A., Roura, S.I., Moreira, M. del R., 2011. Essential Oils as Biopreservatives: Different
676 Methods for the Technological Application in Lettuce Leaves. J. Food Sci. 76, 34–40.
677 https://doi.org/10.1111/j.1750-3841.2010.01880.x

678 Qadri, O.S., Yousuf, B., Srivastava, A.K., 2015. Fresh-cut fruits and vegetables: Critical factors
679 influencing microbiology and novel approaches to prevent microbial risks: A review. Cogent
680 Food Agric. 1, 1–11. https://doi.org/10.1080/23311932.2015.1121606

681 Quitmann, H., Fan, R., Czermak, P., 2014. Acidic organic compounds in beverage, food and feed
682 production, in: Zorn, H., Czermak, P. (Eds.), Biotechnology of Food and Feed Additives. springer,
683 London, pp. 113–114. https://doi.org/10.1007/978-3-662-43761-2

684 Rössle, C., Gormley, T., Butler, F., 2009. Efficacy of Natureseal (R) AS1 browning inhibitor in
685 fresh-cut fruit salads applications, with emphasis on apple wedges. J. Hortic. Sci. Biotecnol.
686 ISAFRUIT Spec. Issue.

687 Shannon, T.C., Pratt, D.E., 1967. Apple polyphenol oxidase activity in relation to various
688 phenolic compounds. J. Food Sci. 32, 479–483.

689 Sperber, W., 2009. Compendium of the Microbiological Spoilage of Foods and Beverages,
690 Compendium of the Microbiological Spoilage of Foods and Beverages.
691 https://doi.org/10.1007/978-1-4419-0826-1

692 Takahashi, H., Kashimura, M., Koiso, H., Kuda, T., Kimura, B., 2013. Use of ferulic acid as a
693 novel candidate of growth inhibiting agent against Listeria monocytogenes in ready-to-eat food.
694 Food Control 33, 244–248. https://doi.org/10.1016/j.foodcont.2013.03.013

695 Wilson, M.D., Stanley, R.A., Eyles, A., Ross, T., 2019. Innovative processes and technologies
696 for modified atmosphere packaging of fresh and fresh-cut fruits and vegetables. Crit. Rev. Food
697 Sci. Nutr. 59, 411–422. https://doi.org/10.1080/10408398.2017.1375892

698 Zduńska, K., Dana, A., Kolodziejczak, A., Rotsztejn, H., 2018. Antioxidant Properties of Ferulic
699 Acid and Its Possible Application. Ski. Pharmacol. Phisiology 31, 332–336.
700 https://doi.org/10.1159/000491755

Zhong, Y., Cavender, G., Zhao, Y., 2014. Investigation of different coating application methods on the performance of edible coatings on Mozzarella cheese. LWT - Food Sci. Technol. 56, 1–8. https://doi.org/10.1016/j.lwt.2013.11.006

Table 1. Low, medium and high concentrations of FA used for each pathogenic strain.

	FA treatment		
Strain	**FA-L** ($g\ L^{-1}$)	**FA-M** ($g\ L^{-1}$)	**FA-H** ($g\ L^{-1}$)
S. Agona	10.0	12.5	15.0
S. Michigan	7.5	10.0	12.5
S. Montevideo	5.0	7.5	10.0
S. Typhimurium	7.5	10.0	12.5
L. monocytogenes 1/2	2.5	5.0	7.5
L. monocytogenes 4b	2.5	5.0	7.5
L. monocytogenes 1/2a	5.0	7.5	10.0

FA-L, lower concentration of ferulic acid; FA-M, medium concentration of ferulic acid; FA-H, higher concentration of ferulic acid tested.

Table 2. Initial quality parameters of apple and melon used for the experiments: pH, total soluble solids (%), and titratable acidity (malic acid for apple, citric acid for melon, mg L^{-1}) (n=21).

Fruit	**pH**	**Total soluble solids** (%)	**Titratable acidity** (mg L^{-1})
Apple	4.6 ± 0.3	14.6 ± 1.2	1.6 ± 0.2
Melon	5.7 ± 0.3	11.3 ± 0.8	1.3 ± 0.1

FW, fresh weight

Table 3. Initial (D0) and final (D7, after 7 d of storage at 10 ºC) populations of pathogenic strains in untreated apple and melon (n=3).

	Apple		**Melon**	
Strain	**Initial population** (log CFU g^{-1})	**Final population** (log CFU g^{-1})	**Initial population** (log CFU g^{-1})	**Final population** (log CFU g^{-1})
S. Agona	6.2 ± 0.1	5.2 ± 0.9	6.2 ± 0.1	7.9 ± 0.1
S.. Michigan	5.5 ± 0.2	7.3 ± 0.1	6.4 ± 0.1	8.3 ± 0.1
S. Montevideo	6.3 ± 0.2	7.4 ± 0.2	6.3 ± 0.1	8.6 ± 0.1
S. Typhimurium	6.5 ± 0.1	7.2 ± 0.5	6.6 ± 0.1	8.6 ± 0.1
L. monocytogenes 1/2	5.6 ± 0.2	7.9 ± 0.5	6.4 ± 0.1	9.5 ± 0.1
L. monocytogenes 4b	5.8 ± 0.1	8.5 ± 0.1	6.3 ± 0.1	9.2 ± 0.1
L. monocytogenes 1/2a	5.8 ± 0.1	8.2 ± 0.1	6.2 ± 0.1	9.3 ± 0.1

Table 4. Population of *L. monocytogenes* cocktail in untreated apple and melon at initial (D0) and final (D7, after 7 d of storage at 10 ºC). Reductions of *L. monocytogenes* populations compared with the population of the untreated samples after FA treatments at different concentrations, in apple and melon (n=3). Different lowercase letters indicate statistically significant differences between treatments in the same storage day and fruit ($p < 0.05$), according to Tukey's HSD test.

		Apple		**Melon**	
		D0	**D7**	**D0**	**D7**
Population (log CFU g^{-1})	**Untreated**	5.9 ± 0.3	7.9 ± 0.5	6.2 ± 0.1	9.3 ± 0.1
Reduction (log)[1]	**FA-1.0**	0.4 ± 0.1 ^{a}	3.7 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	1.0 ± 0.1 ^{a}
	FA-1.5	0.4 ± 0.1 ^{a}	4.1 ± 0.1 ^{b}	0.5 ± 0.2 ^{a}	2.0 ± 0.2 ^{b}
	FA-2.0	0.5 ± 0.1 ^{a}	3.9 ± 0.1 ^{ab}	0.4 ± 0.1 ^{a}	3.3 ± 0.1 ^{c}
	FA-2.5	0.4 ± 0.1 ^{a}	3.7 ± 0.1 ^{a}	0.5 ± 0.1 ^{a}	3.9 ± 0.1 ^{d}

[1]Reduction (log units)$_d$ = (Log N_d/N_0) Eq. 1. Where N_0 is the mean of the population of untreated discs (as a population reference), and N_d is the population of each treatment at sampling date (d) (CFU g $^{-1}$).

Table 5. Firmness values (N) of apple (NS or FA-2.5) and melon (W or FA-2.5) at different storage days (n=30). Values are the mean and the bars represent the standard deviation. Different lowercase letters indicate statistically significant differences between treatments in the same storage day and fruit ($p < 0.05$). Different capital letters indicate statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukey's HSD test.

		Firmness (N)	
Fruit	**Day**	**Control***	**FA-2.5**
	D0	13.84 ± 0.25^{aA}	10.09 ± 0.75^{bA}
	D5	15.49 ± 0.71^{aAB}	8.65 ± 0.58^{bAB}
	D8	19.56 ± 0.22^{aC}	8.07 ± 0.75^{bBC}
Apple	D12	18.14 ± 1.36^{aBC}	6.85 ± 0.17^{bC}
	D0	5.82 ± 0.35^{aA}	5.69 ± 0.17^{aA}
	D3	5.56 ± 0.06^{aA}	5.79 ± 0.20^{aA}
	D5	5.88 ± 0.31^{aA}	5.82 ± 0.35^{aA}
Melon	D7	4.86 ± 0.17^{aB}	4.89 ± 0.15^{aB}

NS, Naturseal ® treatment; FA-2.5, ferulic acid at 2.5 g L^{-1}; W, water.

*Controls: NS for apple, W for melon.

Table 6. Sensory evaluation of fresh-cut apple (NS or FA-2.5, 0 and 8 d) and fresh-cut melon (W or FA-2.5, 0 and 7 d) in a 9-point hedonic scale (n=20). Different lowercase letters indicate statistically significant differences between treatments in the same storage day and fruit ($p < 0.05$) according to Tukey's HSD test.

		Punctuation in 9-point hedonic scale	
Fruit	**Day**	**Control***	**FA-2.5**
Apple	D0	6.9 ± 1.2 [a]	5.2 ± 1.2 [b]
	D8	6.6 ± 1.5 [a]	4.9 ± 1.8 [b]
Melon	D0	7.6 ± 1.1 [a]	7.0 ± 1.8 [a]
	D7	7.1 ± 1.4 [a]	6.4 ± 1.9 [a]

NS, Naturseal ® treatment; FA-2.5, ferulic acid at 2.5 g L^{-1}; W, water.

*Controls: NS for apple, W for melon.

Table 7. Antioxidant capacity values by FRAP and DPPH· methods (AAE· 10^{-2} in g kg^{-1}) and total phenolic content (TPC) (GAE· 10^{-2} in g kg^{-1}) of apple (NS or FA-2.5) and melon (W or FA-2.5) at different storage days (n=3). Values are the mean and the bars represent the standard deviation. Different lowercase letters indicate statistically significant differences between treatments in the same storage day and fruit ($p < 0.05$). Different capital letters indicate statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukey's HSD test.

Fruit	Day	**FRAP** (· 10^{-2}, g kg^{-1})		**DPPH**· (· 10^{-2}, g kg^{-1})		**TPC** (· 10^{-2}, g kg^{-1})	
		Control*	**FA-2.5**	**Control***	**FA-2.5**	**Control***	**FA-2.5**
Apple	D0	169.06 ± 36.08 ^{aA}	51.06 ± 4.05 ^{bA}	151.90 ± 16.11 ^{aA}	51.47 ± 8.47 ^{bA}	186.11 ± 15.32 ^{aA}	67.54 ± 16.11 ^{bA}
	D5	142.51 ± 10.95 ^{aAB}	45.19 ± 3.14 ^{bA}	135.78 ± 8.07 ^{aAB}	48.15 ± 4.03 ^{bAB}	159.50 ± 16.11 ^{aAB}	60.72 ± 8.07 ^{bA}
	D8	134.97 ± 4.69 ^{aAB}	44.01 ± 2.10 ^{bAB}	116.82 ± 3.29 ^{aB}	45.65 ± 2.14 ^{bAB}	147.76 ± 28.80 ^{aAB}	60.57 ± 3.29 ^{bA}
	D12	110.75 ± 6.46 ^{aB}	36.41 ± 2.53 ^{bB}	88.65 ± 5.26 ^{aC}	36.50 ± 1.25 ^{bB}	119.80 ± 8.11 ^{aB}	55.73 ± 5.26 ^{bA}
Melon	D0	8.64 ± 0.24 ^{bAB}	14.24 ± 0.70 ^{aAB}	2.63 ± 0.60 ^{bA}	14.99 ± 0.22 ^{aA}	14.03 ± 3.25 ^{bA}	44.61 ± 8.29 ^{aA}
	D3	8.81 ± 0.04 ^{bA}	13.46 ± 0.28 ^{aB}	2.38 ± 0.15 ^{bA}	15.30 ± 0.55 ^{aA}	10.27 ± 1.09 ^{bAB}	39.91 ± 2.29 ^{aA}
	D5	8.43 ± 0.09 ^{bBC}	13.58 ± 0.12 ^{aB}	3.20 ± 0.24 ^{bA}	15.45 ± 0.59 ^{aA}	9.64 ± 1.91 ^{bAB}	39.81 ± 1.82 ^{aA}
	D7	8.16 ± 0.06 ^{bC}	14.72 ± 0.05 ^{aA}	4.16 ± 0.14 ^{bB}	12.48 ± 0.42 ^{aB}	7.79 ± 0.54 ^{bB}	36.61 ± 2.08 ^{aA}

NS, Naturseal® treatment; FA-2.5, ferulic acid at 2.5 g L^{-1}; W, water; TPC, total phenolic content; AAE, ascorbic acid equivalents; GAE, gallic acid equivalents.

*Controls: NS for apple, W for melon.

Figure 1. Population changes in counts of *S.* Agona (**A**), *S.* Montevideo (**B**), *S.* Gaminara (**C**), *S.* Typhimurium (**D**), *L. monocytogenes* serovar 1/2 (**E**), *L. monocytogenes* serovar 4b (**F**), and *L. monocytogenes* serovar 1/2a (**G**), in control treatment (CT), or in FA at low concentration (FA-L), at a medium concentration (FA-M) or at a high concentration (FA-H) compared to untreated samples, after 7 d of storage (D7) at 10 ˚C, in apple (■) and melon (■) discs. Values are the mean ± standard deviation (n=3). Within the same fruit, different letters mean statistically significant differences between treatments ($p < 0.05$) according to Tukey's HSD test.

Figure 1

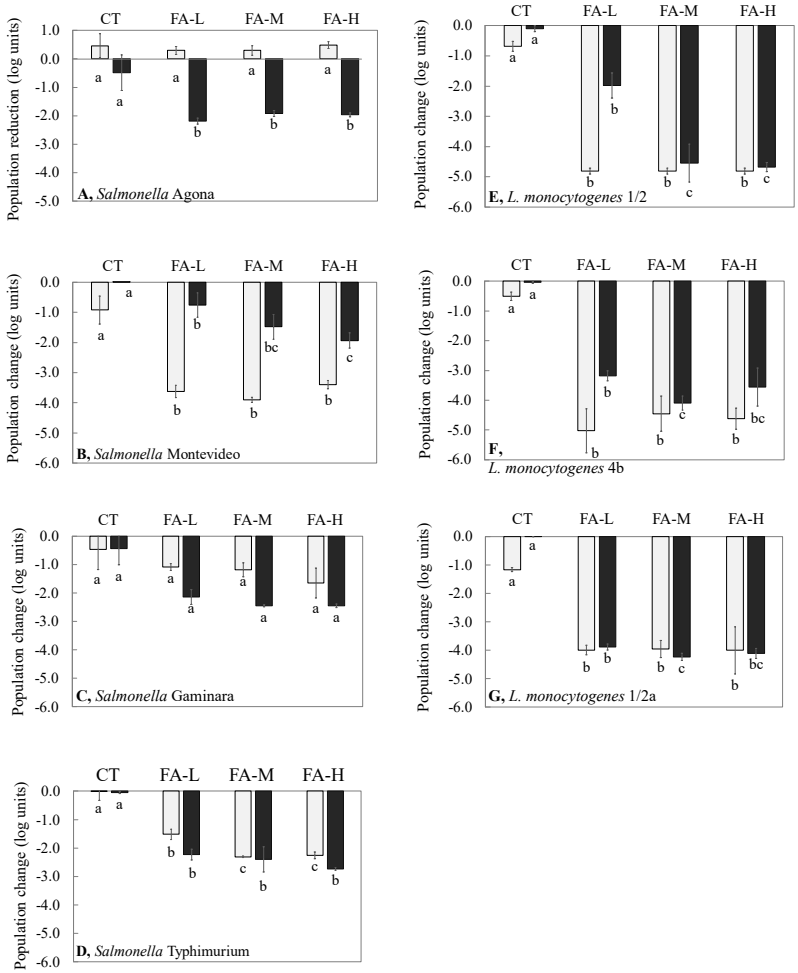


Figure 2. Population changes in counts of (**A**) *S. enterica*, and (**B**) *L. monocytogenes*, in comparison to untreated sample in apple (■) and melon (■) discs, treated with different FA concentrations (2.5, 5.0, and 7.5 g L^{-1}) by immersion (I) or by spray (S) after 7 d of storage (D7) at 10 ˚C. Values are the mean ± standard deviation (n=3). Within the same fruit, different letters mean statistically significant differences between treatments ($p < 0.05$), according to Tukey's HSD test.

Figure 2

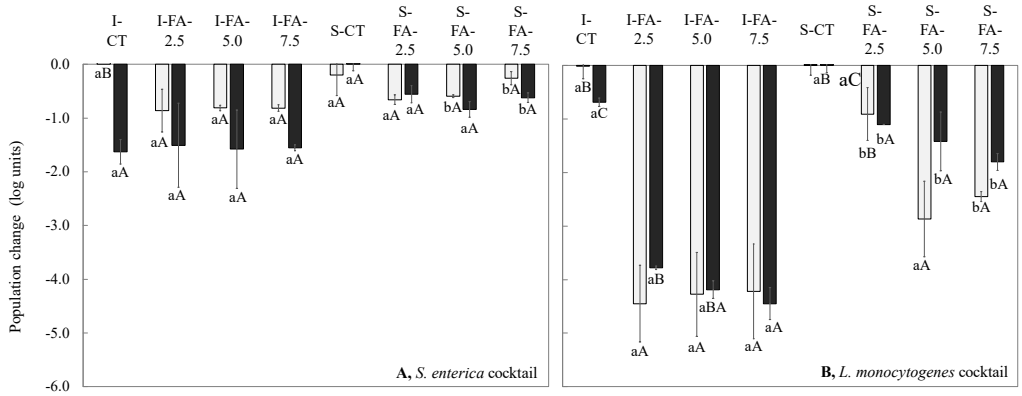


Figure 3. Browning index (BI) in Naturseal ® (NS, ■) and 2.5 g L^{-1} FA (FA-2.5, ■) treated fresh-cut apple (**A**), and total color difference (TCD) in water control (W, ■) and 2.5 g L^{-1} FA (FA-2.5, ■) treated fresh-cut melon (**B**) in trial 4 during storage at 4 ºC. Values are the mean ± standard deviation (n=3). Different lowercase letters mean statistically significant differences between treatments for the same day ($p < 0.05$). Different capital letters mean statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukey's HSD test.

Figure 3

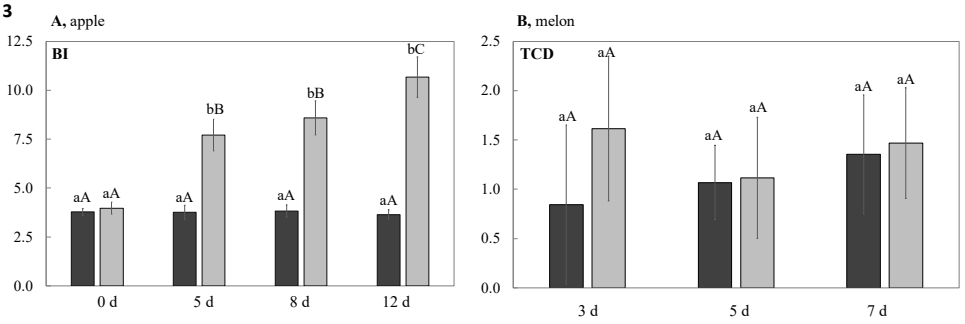
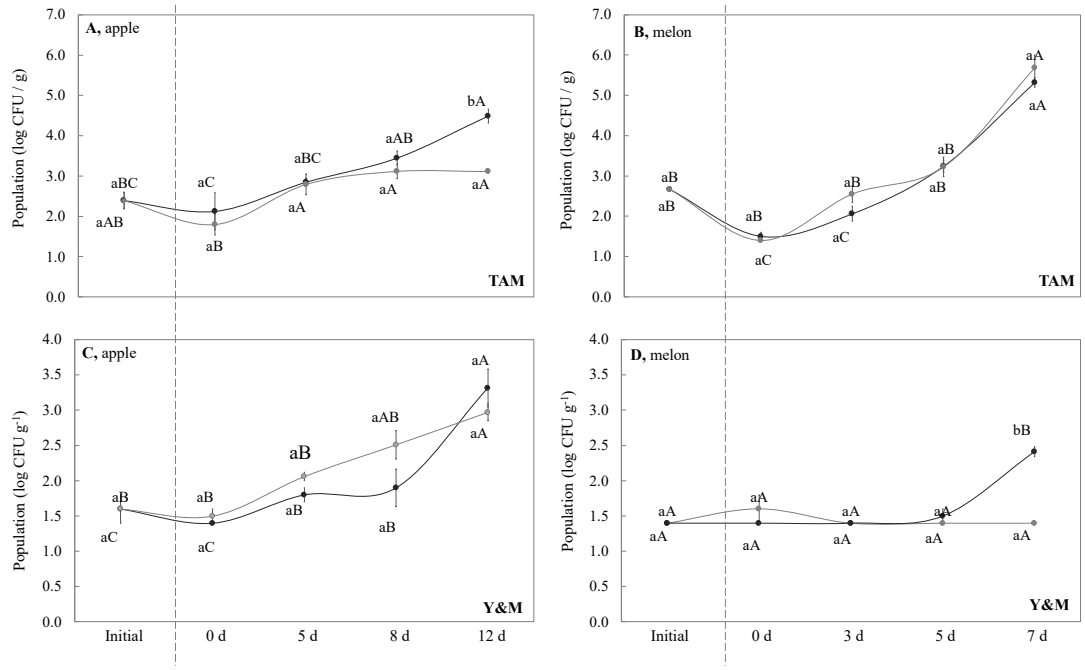


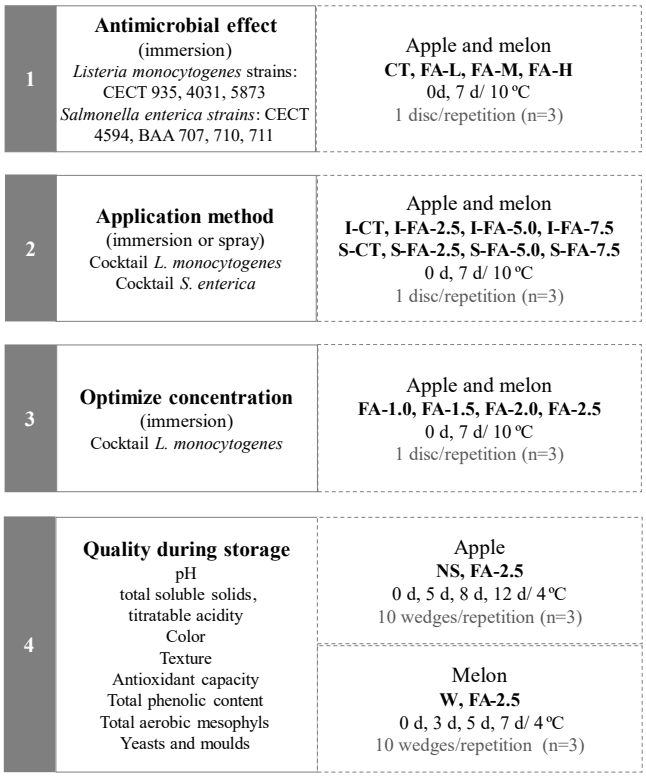
Figure 4. Counts of total aerobic mesophylls (TAM, A and B) and yeasts and molds (Y&M, **C and D**) populations of Naturseal ® (NS, ■) and 2.5 g L^{-1} FA (FA-2.5, ■) treated fresh-cut apple (**A and C**) and of water control (W, ■) and 2.5 g L^{-1} FA (FA-2.5, ■) treated fresh-cut melon (**B and D**) in trial 4 during storage at 4 ºC. Values are the mean ± standard deviation (n=3). Different lowercase letters mean statistically significant differences between treatments for the same day ($p < 0.05$). Different capital letters mean statistically significant differences between days within the same treatment ($p < 0.05$) according to Tukey's HSD test

Figure 4



SUPPLEMENTARY 1. Experimental design

Supplementary Figure 1



SUPPLEMENTARY 2. Changes in color values L*, a*, b* in NS (■) and FA-2.5 (■) fresh-cut apple (**A**), and L*, a*, b* in W (■) and FA-2.5 (**-**) fresh-cut melon (**B**) in trial 4 during storage at 4 ºC. Values are the mean ± standard deviation (n=3). Different lowercase letters mean statistically significant differences between treatments for the same day ($p < 0.05$). Different capital letters mean statistically significant differences between days within the same treatment ($p < 0.05$).

Supplementary figure 2