Effect of ripeness stage during processing on *Listeria monocytogenes* growth on fresh-cut 'Conference' pears.

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ABSTRACT

There are several factors that affect the shelf life of fresh-cut fruit, including the cultivar, the ripeness stage of the fruit during processing and the fruit's storage atmosphere and temperature. The effect of fruit ripeness during processing on the survival and growth of *Listeria monocytogenes* on fresh-cut 'Conference' pear slices at different temperatures (5, 10 and 20 °C) was studied. The four ripeness stages studied in this work (assessed by a fruit's firmness) were mature-green (54-60 N), partially ripe (43-53 N), ripe (31-42 N) and overripe (< 31 N). In our studies, pH, acidity and soluble solids content did not significantly change during conditioning at 20 °C. *L. monocytogenes* grew under all experimental conditions, showing an increase of approximately 2 log CFU g^{-1} after 8 days of storage at 5 °C. There were significant differences in the *L. monocytogenes* population between different ripeness stages at the end of the experiments at 10 and 20 °C. Regardless of the ripeness stage of a fresh-cut pear, the growth potential of *L. monocytogenes* increased with increasing temperature. A pear's ripeness stage during processing is an important consideration to ensure the quality of a fresh-cut pear, but it is not as important for preventing *L. monocytogenes* growth at common storage temperatures.

Keywords: *Listeria monocytogenes*, fresh-cut pear, ripeness stage, growth

1. Introduction

In recent years, the consumption of fresh-cut fruits and vegetables has quickly increased. This increase was motivated by fruit's desirable qualities such as its freshness, low-calorie composition, nutritional content and convenience. The average fruit and vegetable consumption in the European Union (EU) and the United States is 6 and 30 kg/year/person, respectively. However, the consumption for each country within the EU varies. For example, in the United Kingdom the average consumption is 20 kg/year/person, followed by France and Italy at 12 and 8 kg/year/person, respectively. Despite the low consumption of fresh-cut fruits and vegetables in Spain (3 kg/year/person, Anonymous, 2014), the production these fruits and vegetables has continuously increased, from 36700 to 60169 to 129637 ton in 2004, 2007 and 2013, respectively. Some fresh-cut fruits, such as apples, pineapples, melons, mangos and fruit mixes, are being sold in Spanish markets; however, their sales are low compared with vegetable sales (FEPEX, 2013).

Pears (*Pyrus communis L.*) have low protein and lipid contents and are rich in sugars such as fructose, sorbitol, and sucrose and low in glucose. Pears also contain micronutrients, such as vitamins, minerals and antioxidants. Lleida province, located in Catalonia (Spain), is the first province in Spain to produce pears. In 2011, 502434 tons of pears were produced in Spain, 49.8 % of which were produced in Lleida (Magrama, 2012). Moreover, in 2012 175493 ton of common pears produced in Lleida were distributed as follows: 22638 ton of 'Blanquilla', 65810 ton of 'Conference' and 25601 ton of 'Llimonera' (DAAM, 2012). The production of fresh-cut pears can potentially increase profits for companies by serving as an alternative to fresh fruit sold at markets.

Some important factors that affect the shelf life of fresh-cut fruit include the specific variety of the fruit, the fruit's stage of ripeness at the cutting step, and the fruit's storage atmosphere and temperature (Gorny et al., 2000). 'Conference' pears are the most produced variety in Lleida. These pears can be stored at low temperatures in a controlled atmosphere for an extended period of time (Nguyen et al., 2007). Furthermore, among the different varieties of pear, 'Conference' pears are the most suitable for fresh-cut fruit production (Arias et al., 2008; Soliva-Fortuny et al., 2004; Colás-Medà et al. (unpublished data)). Several studies have been carried out to determine the optimal ripeness stage for pear processing, based on a pear's firmness. Soliva et al. (2004) demonstrated that partially ripe (firmness: 44 ± 3.2 N) 'Conference' pears were the most suitable for processing. Gorny et al. (2000) determined that 'Barlett' pears were ideal for processing when they were partially ripe (44 to 58 N). Moreover, Oms-Oliu et al. (2009) also studied the effect of the ripeness of 'Flor de Invierno' pears on the growth of indigenous microbiota. They found that rapid microbial growth occurred on ripe pears (36.1 N), and partially ripe pears (43.3 N) were suitable for conservation while gathering desired sensory attributes.

Two key processing steps for preserving fruit are the removal of the peel or rind and the cutting of the fruit. The protective barrier is removed during the processing of fresh-cut fruits and vegetables, which makes the produce especially vulnerable to microbial contamination and colonization and increases the risk of fresh-cut produce becoming a health hazard (Leverentz et al., 2001). In the last few years, there have been several outbreaks linked to the consumption of contaminated fruits and vegetables. *Salmonella* spp. and *E. coli* O157:H7 outbreaks have been linked to the consumption of cantaloupe, watermelon, mango, tomato, papaya and fruit salads

(CDC; Harris et al., 2003). Although *Listeria monocytogenes* outbreaks have only been linked to the consumption of cantaloupes and tomatoes (CDC, 2014; Harris et al., 2003), those incidences resulted in a high mortality rate. *L. monocytogenes* is a gram-positive bacterium, a facultative anaerobic and an important foodborne pathogen. There are 13 serotypes of *L. monocytogenes*, but 90 % of human infections are usually associated with three specific serotypes: 1/2a, 1/2b and 4b. This microorganism can grow at temperatures between -0.4 °C and 45 °C, with 37 °C being the optimal growth temperature. In addition, *L. monocytogenes* can grow anywhere between pH 4.4 and pH 7.0, depending on the temperature (Walker and Stringer, 1987). The growth of *L. monocytogenes* under refrigerated and ambient conditions has been evaluated in several studies on fruits, including apples (Alegre et al., 2010a; Conway et al., 2000), peaches (Alegre et al., 2010b), strawberries (Flessa et al., 2005), persimmons (Uchima et al., 2008) and melons, watermelons and papayas (Penteado and Leitão, 2004; Uchima et al., 2008); however, no studies have been carried out on fresh-cut pears.

The objective of the present study was to determine the effect of fruit ripeness during processing on the survival and growth of *L. monocytogenes* on fresh-cut pear slices stored at various temperatures.

2. Materials and Methods

2.1. Fruit

'Conference' pears (*Pyrus communis* L. cv. Conference) were acquired from a local shipper in the city of Lleida (Catalonia, Spain). The fruits were stored at 0 °C until use. The pears were ripened by incubation at 20 °C, for a maximum of 72 h, until the desired ripeness was achieved (Soliva-Fortuny et al., 2004). In this study, the ripeness

115 stage of a pear was determined by its firmness. Flesh firmness was measured on
116 opposite sides of each fruit with a penetrometer (Effegi, Mila, Italy) equipped with a
117 probe 8 mm in diameter. Once the desired firmness values were achieved, the pears
118 were incubated at 0 °C overnight. The ripeness stage categorizes in this work were 54-
119 60 N (mature-green), 43-53 N (partially ripe), 31-42 N (ripe) and < 31 N (overripe) and
120 were determined by sampling 10 fruits for each category.

121 2.2. Microorganisms and preparation of cell suspensions

122 For this study, *Listeria monocytogenes* serovar 1/2a was isolated from commercial
123 fresh-cut Iceberg lettuce (Abadias et al., 2008). *L. monocytogenes* was grown overnight
124 at 37 ± 1 °C in tryptone soy broth (TSB, Oxoid, UK) supplemented with 6 g L^{-1} of yeast
125 extract (tryptone yeast extract soy broth, TYSEB). Bacterial cells were harvested by
126 centrifugation at 9820 x g and 10 °C for 10 min and then resuspended in saline
127 peptone (SP; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone). The concentration was estimated using
128 a spectrophotometer set at λ = 420 nm and a standard curve. For inoculum
129 preparation, an aliquot of the foodborne pathogen suspension was added to deionized
130 water to obtain approximately 10^5 CFU mL^{-1}. The inoculum concentration was
131 determined by plating dilutions onto Palcam agar (Palcam Agar Base with selective
132 supplement, Biokar Diagnostics, Beauvais, France) and incubating the plates at 37 ± 1
133 °C for 48 h.

134 2.3. Inoculation of fruits and testing of the packaging and storage conditions

135 Prior to the experiments, pears were washed with running tap water and dried by
136 hand with absorbent paper to eliminate plant debris and pesticide residues. Then the
137 pears' surfaces were disinfected with 70 % ethanol. Pears were peeled and cut into 10
138 wedges using a handheld apple corer and slicer. Afterwards, pear wedges were

139 inoculated by immersion into an *L. monocytogenes* suspension (1:2 w/v) shaken at 150
140 rpm for 2 min (Abadias et al., 2014 and Alegre et al., 2013). Next, the liquid was
141 drained off, and the wedges were left to air-dry in a biosafety cabinet. Fresh-cut pears
142 (150 g) were placed on covered polypropylene trays (500 mL) in ambient air (21 % O_2,
143 0 % CO_2). Once packed, the trays of pears were stored at 20 ± 1 °C, 10 ± 1 °C and 5 ± 1
144 °C. The pears stored at 5 and 10 °C were examined on the day of inoculation and after
145 2, 5 and 8 days. The samples stored at 20 °C were examined on the day of inoculation
146 and after 5, 10, 22, 29 and 45 h.
147 2.4. Physicochemical analyses of fresh-cut pears
148 Before inoculation, the pH of the fresh-cut pears was measured using a pH meter
149 Model GLP22 (Crison Instruments S.A., Barcelona, Spain) with a penetration electrode
150 (5231 Crison). After the pH reading, the pears were squeezed, and the soluble solids
151 content (SCC) was determined using a handheld refractometer at 20 °C (Atago CO.,
152 LTD, Japan). The results were expressed in °Brix. To measure the titratable acidity (TA),
153 10 mL of pear juice was diluted with 10 mL of deionized water and then titrated with a
154 0.1 N sodium hydroxide (NaOH) solution to pH 8.1. The results were calculated as g of
155 malic acid per litre of solution.
156 2.5. Enumeration and detection of *L. monocytogenes*
157 The population of *L. monocytogenes* was determined for three sample trays for each
158 ripeness stage at each sampling time and temperature. At each sampling time, 10 g of
159 fruit was placed in a sterile plastic bag (400 mL, BagPage, Interscience, BagSystem, St
160 Nom La Breteche, France), and 90 mL of buffered peptone water (BPW, Oxoid, LTD,
161 Basingstoke, Hampshire, England) was added. This mixture was homogenized in a
162 stomacher blender at 250 impact s^{-1} for 90 s (IUL, Masticator, Spain). Aliquots of the

163 mixture were serially diluted into SP, the surface was placed onto Palcam agar and the
164 agar plates were incubated at 37 ± 1 °C for 48 h. The results were expressed as colony
165 forming units (CFU) of *L. monocytogenes* per gram of pear. The data were plotted on a
166 decimal logarithm (log) scale. Each experiment was performed in duplicate.
167 Moreover, the growth potential of *L. monocytogenes* in each ripeness stage was
168 assessed by comparing the difference between the log CFU g^{-1} at the beginning
169 (corresponding to the end of the processing, time 0) and end (at 5 and 10 °C: day 8;
170 and at 20 °C: 45 h) of the assay (Beaufort, 2011). According to Regulation (EC) No.
171 2073/2005, if the growth potential is higher than 0.5 log CFU g^{-1}, the food is assumed
172 to be capable of facilitating the growth of *L. monocytogenes*.
173 2.6. Statistical analysis
174 Data of *L. monocytogenes* growth (log CFU g^{-1}) and quality parameters were analysed
175 using general linear model analysis with JMP8 software (SAS Institute, Cary, NC, USA).
176 Statistical significance was judged at the level of $P < 0.05$. When the analysis was
177 statistically significant, the least significance difference (LSD) test for separation of
178 means was used.
179
180 **3. Results**
181 3.1. Physicochemical parameters of fresh-cut 'Conference' pears
182 Different pear batches were used for each temperature experiment; therefore, the
183 physicochemical quality parameters are shown for each temperature. At 5 °C, the
184 mean firmness values for 54-60 N (mature-green), 43-53 N (partially ripe), 31-42 N
185 (ripe) and < 31 N (overripe) were 56.3 ± 3.1, 48.1 ± 2.2, 37.4 ± 3.3 and 20.2 ± 3.9 N,
186 respectively (Table 1). The overripe pears had the lowest pH (4.85 ± 0.28), while the

187 highest pH was observed in partially ripe pears (pH 5.17 ± 0.14). SSC values were not
188 significantly different among the ripeness stages studied (14.7-15.1 °Brix). However,
189 there were significant differences ($P< 0.05$) in TA, with the ripe stage presenting the
190 highest TA (1.60 ± 0.36 g malic acid L^{-1}).
191 The mean firmness values of mature-green, partially ripe, ripe and overripe pears were
192 56.8 ± 3.1, 48.2 ± 3.2, 36.1 ± 3.8 and 21.2 ± 4.5 N, respectively, at 10 °C (Table 2).
193 Mature-green pears had the lowest pH (4.70 ± 0.24), and ripe pears had the highest pH
194 (5.17 ± 0.28). The SSC was not affected by the ripeness stage (14.6-15.3 °Brix). Mature-
195 green pears had the highest TA (1.56 ± 0.26 g malic acid L^{-1}), while ripe and overripe
196 pears showed the lowest TAs (1.18 ± 0.18 and 1.21 ± 0.27 g malic acid L^{-1}, respectively).
197 In the 20 °C experiments, the mean firmness of mature-green, partially ripe, ripe and
198 overripe pears were 58.6 ± 3.7, 50.6 ± 2.6, 36.0 ± 2.6 and 20.6 ± 2.1 N, respectively
199 (Table 3). The pH values were not significantly different ($P > 0.05$) between the
200 ripeness stages studied (4.91-5.04). Overripe pears had the lowest SSC (14.6 ± 0.5
201 °Brix), while ripe pears had the highest SSC (15.1 ± 0.5 °Brix). Overripe pears had the
202 highest TA (1.05 ± 0.15 g malic acid L^{-1}), while partially ripe pears showed the lowest
203 TA (0.84 ± 0.15 g malic acid L^{-1}).
204 3.2. Effect of the ripeness stage on the growth of *L. monocytogenes* in fresh-cut pears
205 3.2.1. Survival of *L. monocytogenes* on fresh-cut pears stored at 5 °C.
206 The initial *L. monocytogenes* population in fresh-cut pears was 3.3 ± 0.1 log CFU g^{-1}
207 (Figure 1). The overripe fresh-cut pears had significantly lower counts of *L.*
208 *monocytogenes* than pears of all of the other ripeness stages, with 3.3 ± 0.1 and 4.2 ±
209 0.3 log CFU g^{-1} after 2 and 5 days of storage at 5 °C, respectively. After 8 days of
210 storage at 5 °C, there were no significant differences among the *L. monocytogenes*

211 population in fresh-cut pears processed at different ripeness stages. All final
212 population counts were between 5.2 ± 0.4 and 5.6 ± 0.4 log CFU g^{-1}.

213 3.2.2. Survival of *L. monocytogenes* on pear slices stored at 10 °C

214 After inoculation, all the fresh-cut pears had an initial *L. monocytogenes* population of
215 3.4 ± 0.2 log CFU g^{-1}, regardless of the ripeness stage of the pear. After 5 days of
216 storage at 10 °C, the *L. monocytogenes* population was significantly higher in the
217 overripe fresh-cut pears (7.0 ± 0.4 log CFU g^{-1}), while mature-green pears had the
218 lowest pathogen population (6.4 ± 0.4 log CFU g^{-1}). At the end of the experiment (8
219 days), the populations were 6.8 ± 0.5, 6.9 ± 0.4, 7.3 ± 0.2 and 7.5 ± 0.4 log CFU g^{-1}, in
220 the mature-green, partially ripe, ripe and overripe pears, respectively. Significant
221 differences were observed in the overripe and mature-green pears. The maximum
222 population of *L. monocytogenes* (7.5 ± 0.4 log CFU g^{-1}) was observed in the overripe
223 fresh-cut pears after 8 days of storage.

224 3.2.3. Survival of *L. monocytogenes* on pear slices stored at 20 °C

225 The growth rate of *L. monocytogenes* on fresh-cut pear slices stored at 20 °C was
226 higher than that on slices stored at 5 and 10 °C (Figure 3). Moreover, the duration of
227 the 20 °C experiment was shorter than that of the 5 and 10 °C experiments. After
228 inoculation, fresh-cut pears presented an initial *L. monocytogenes* population of 3.3 ±
229 0.1 log CFU g^{-1}. At the end of the evaluation, around 2 days (exactly 45 h), the
230 population of *L. monocytogenes* was 7.3 ± 0.1, 7.0 ± 0.4, 7.5 ± 0.1 and 7.6 ± 0.1 log CFU
231 g^{-1} in the mature-green, partially ripe, ripe and overripe, respectively; the partially ripe
232 pears had the lowest population (7.0 ± 0.4 log CFU g^{-1}).

233 3.3. Growth potential (δ) of *L. monocytogenes* on fresh-cut pears

234 Table 4 shows the growth potential (δ) of *L. monocytogenes* 1/2a at different storage
235 temperatures. As expected, the δ values were higher for higher storage temperatures.
236 After 2 days at 5 °C, the highest δ of *L. monocytogenes* (0.42 log CFU g^{-1}) was observed
237 in mature-green pears, and the lowest δ (0.18 log CFU g^{-1}) was observed in overripe
238 pears. In contrast, at 10 °C the highest δ of *L. monocytogenes* (1.77 log CFU g^{-1}) was
239 found in overripe pears, and the lowest (1.57 log CFU g^{-1}) in mature-green pears. At 20
240 °C, the δ of *L. monocytogenes* was similar (4.41 log CFU g^{-1}) in ripe and overripe pears,
241 while partially ripe pears had the lowest δ value (3.84 log CFU g^{-1}) after 2 days. At the
242 end of the 5 °C experiment (8 days) at 5 °C mature-green and ripe pears had the
243 highest δ values (2.41 and 2.44 log CFU g^{-1}, respectively), and partially ripe and
244 overripe had the lowest δ values (2.11 and 2.19 log CFU g^{-1}, respectively). For the 10 °C
245 experiments, the pears with low firmness (overripe) had the highest δ values of *L.*
246 *monocytogenes* (4.40 log CFU g^{-1}), while the firmest pears (mature-green) had the
247 lowest δ value of 3.48 log CFU g^{-1} after 8 days of storage. The growth potential of *L.*
248 *monocytogenes* after 8 days of storage (normal shelf life) under reasonable conditions
249 (5 °C) was greater than 0.5 log CFU g^{-1} regardless of the ripeness stage of the pear.
250 These results confirm that fresh-cut 'Conference' pears are able to facilitate the
251 growth of *L. monocytogenes*.
252
253 **4. Discussion**
254 To our knowledge, this is the first study that assesses the growth of *L. monocytogenes*
255 on fresh-cut 'Conference' pears at different ripeness stages and storage temperatures.
256 The ripeness stage was determined based on the firmness of the pear (Gorny et al.,
257 2000; Oms-Oliu et al., 2009; Soliva-Fortuny et al., 2004).

258 The ‘Conference’ pears used in this study had a pH between 4.70 and 5.55, a SSC
259 between 13.8 and 15.8 °Brix and a TA between 0.84 and 1.60 g malic acid L^{-1}. Our
260 studies showed that pH, acidity and soluble solids content did not significantly change
261 during the 20 °C incubation. The slight differences observed were probably because
262 different batches of pears were used for different sets of experiments. In accordance
263 with our findings, Cano-Salazar et al. (2012; 2013) did not observe significant changes
264 in the SSC, TA and colour of different peach and nectarine cultivars incubated at 20 °C
265 for 3 days.
266 Our results confirm that the serovar 1/2a strain of *L. monocytogenes* can grow on
267 fresh-cut ‘Conference’ pears with a firmness from 59 N to less than 20 N at storage
268 temperatures of 5, 10 and 20 °C. Regardless of the pear’s ripeness, *L. monocytogenes*
269 was able to grow on the pears even when they were stored at 5 °C, with an increase in
270 the population of approximately 2 log CFU after 8 days. Notably, for the 5 °C batch,
271 overripe pears had the lowest pH (4.85 ± 0.28), and the growth of *L. monocytogenes*
272 on overripe pears was significantly lower than growth on pears at other ripeness
273 stages after 2 and 5 days of storage. However, these differences were not observed
274 after 8 days. The growth of *L. monocytogenes* on other fresh-cut fruits at refrigeration
275 conditions has been studied, and large differences have been observed for certain fruit
276 varieties. Alegre et al. (2010b) found that the *Listeria innocua* population increased by
277 0.4 log CFU on ‘Elegant Lady’ peach plugs (pH 3.73 ± 0.28) after 14 days at 5 °C. On the
278 contrary, Alegre et al. (2010a) found that the *L. innocua* population steadily declined in
279 ‘Granny Smith’ apples (pH 3.32 ± 0.13) and exhibited a more drastic decline from 5.1
280 log CFU $plug^{-1}$ to 1.7 log CFU $plug^{-1}$ in ‘Shampion’ (pH 4.44 ± 0.26) after 14 days. In
281 ‘Golden Delicious’ apples (pH 4.16 ± 0.25), there was an initial drop in the *L. innocua*

282 population, and then the population increased to the inoculum's level at the end of the
283 5 °C experiment. Conway et al. (2000) found that the *L. monocytogenes* population on
284 'Delicious' apples (pH 4.7) did not increase, but the bacteria survived at this
285 temperature throughout the 12-day study. As for strawberries, Flessa et al. (2005)
286 observed a reduction of less than 1 log CFU of *L. monocytogenes* (pH 3.7) after 7 days
287 of storage at 4 °C.
288 At 10 °C, the population of *L. monocytogenes* on fresh-cut pears processed at different
289 ripeness stages increased to values between 3.4 ± 0.2 and 4.0 ± 0.2 log CFU g^{-1} after 8
290 days of storage. There were significant differences between the growth on mature-
291 green and overripe pears, with lower counts on mature-green pears, which had the
292 lowest pH (4.70 ± 0.24) and highest TA (1.56 ± 0.26 g L^{-1} of malic acid L^{-1}). Similar
293 results have been observed on fresh-cut 'Crimson Sweet' watermelons (pH 5.50) in
294 which *L. monocytogenes* presented an increase of 3.5 log CFU after 7 days at 10 °C
295 (Penteado and Leitão, 2004). Uchima et al. (2008) studied two varieties of
296 persimmons, 'Fuyu' and 'Rama Forte', with pH values of 6.3 and 5.5, respectively. In
297 both varieties, there was an increase of 3.5 and 4.0 log CFU in the *L. monocytogenes*
298 population on 'Rama Forte' and 'Fuyu', respectively after 9 days of storage at 10 °C.
299 Higher increases have been observed on fresh-cut melons. Leverentz et al. (2003)
300 observed that the population of *L. monocytogenes* in 'honeydew' melon (pH 5.8)
301 increased by 4.5 log CFU after 7 days at 10 °C. Penteado and Leitão (2004) observed an
302 increase of 6 and 7 log CFU in the *L. monocytogenes* population in 'Valenciano
303 amarelo' melons (pH 5.87) after 7 and 2 days, respectively, at 10 and 20 °C. In contrast,
304 on fresh-cut 'Red delicious' apples (pH 4.4) stored at 10 °C, Leverentz et al. (2006)
305 observed only a slight increase (0.6 log CFU) in the population of *L. monocytogenes*.

306 Similarly, Conway et al. (2000) found that the *L. monocytogenes* population on
307 'Delicious' apples increased by approximately 1.7 log CFU (pH 4.7) after 12 days.
308 At 20 °C, the population of *L. monocytogenes* on fresh-cut pears processed at different
309 ripeness stage increased rapidly to between 3.6 and 4.3 log CFU g^{-1} after 2 days of
310 storage. The highest increase was observed in ripe and overripe pears, reaching 7.5 log
311 CFU g^{-1} after 45 h of storage. Similar results were observed in fresh-cut 'Crimson
312 Sweet' watermelons (pH 5.50), in which the *L. monocytogenes* population increased by
313 4.0 log units after 4 days at 20 °C (Penteado and Leitão, 2004). In previous studies, *L.*
314 *innocua* grew to 6.9 log CFU $plug^{-1}$ on 'Golden Delicious' apples after 2 days, which
315 corresponded to an increase of approximately 2.2 log CFU (Alegre et al., 2010a).
316 Conway et al. (2000) found that the *L. monocytogenes* population increased by
317 approximately 2.7 log CFU after 6 days of storage at 20 °C on 'Delicious' apples (pH
318 4.7). The population of *L. innocua* on 'Elegant Lady' peaches stored at 20 °C increased
319 by approximately 2.8 log CFU after 6 days. In fresh-cut 'Valenciano amarelo' melons
320 (pH 5.87), the population of *L. monocytogenes* increased by 7 log CFU after 4 days at
321 20 °C (Penteado and Leitão, 2004). Uchima et al. (2008) observed an increase of 6.1
322 and 5.1 log CFU in the *L. monocytogenes* population after 41 h at 20 °C in 'Fuyu' and
323 'Rama Forte' persimmons, respectively.
324 At 10 and 20 °C, the increase in the *L. monocytogenes* population on fresh-cut pears
325 was similar to that of fresh-cut watermelons (pH 5.50) (Penteado and Leitão, 2004).
326 Pears and watermelons have a similar pH values (pH 5.5). The pH values of apples,
327 strawberries and peaches were lower than the pH of pears, with values ranging from
328 2.9 to 4.5, 3.0 to 3.6 and 3.5 to 5.0, respectively. In contrast, the pH of melons,
329 watermelons and persimmons were greater than or equal to the pH of the pears, with

values ranging from 6.2 to 6.7, 5.2 to 5.8 and 5.4 to 5.8, respectively. By comparing growth results, we could link the higher pH of the fresh-cut fruit matrix with greater growth in the *L. monocytogenes* population. However, other intrinsic factors, such as water activity, redox potential, availability of nutrients, antimicrobial agents, etc. could play an important role in the behaviour of *L. monocytogenes* on minimally processed fruits.

At the end of the experiment, the growth of *L. monocytogenes* was only influenced by the ripeness stage of pears stored at 10 and 20 °C. At 10 °C, the growth of *L. monocytogenes* was significantly higher on overripe pears than on mature-green pears. This difference in growth could be due to the lower TA of the overripe pears (1.21 ± 0.27 g malic acid L^{-1}) compared with the TA of the mature-green pears (1.56 ± 0.26 g malic acid L^{-1}). At 20 °C, a higher increase was observed in the ripe and overripe pears, reaching a population of 7.5 log CFU g^{-1} at the end of the storage experiments, while a smaller increase was observed in partially ripe pears. Oms-Oliu et al. (2009) studied the effect of ripeness during processing on the shelf life of fresh-cut 'Flor de Invierno' pears and determined its effect on total mesophilic aerobic bacteria, yeast and mould populations. They observed that counts of aerobic mesophilic microorganisms attained at the stationary phase (*A*) were only significantly different for mature-green (65.2 N) fresh-cut pears. In addition, the maximum growth rate and *A* values increased as the ripeness stage advanced. For example, the *A* value for aerobic mesophilic microorganisms in mature-green (65.2 N) fresh-cut pears was 3.2 log CFU g^{-1}, whereas in ripe (36.1 N) fresh-cut pears the A value was 4.7 log CFU g^{-1}. Alegre et al. (2010a) concluded that storage temperature has a major impact on maintaining low levels of foodborne pathogen populations in artificially contaminated

fresh-cut apples. Our results confirm that when the storage temperature increases the growth potential of *Listeria monocytogenes* on fresh-cut 'Conference' pears also increases. For example, in partially ripe pears, the growth potential (δ) of *L. monocytogenes* after 2 days of storage at 5, 10 and 20 °C was 0.36, 1.73 and 3.84 log CFU g^{-1}, respectively.

Our study confirms that 'Conference' pears are an ideal substrate for *L. monocytogenes*. Even though fruit ripeness is a very important consideration for maintaining a pear's quality during its shelf life, it is not an important parameter for preventing *L. monocytogenes* growth at 5 °C. In general, no correlation between a pear's ripeness stage and its *L. monocytogenes* growth was found. We observed that at 5 °C, growth of *L. monocytogenes* at different pear ripeness stages was not significantly different. However, when the storage temperature was harsher (10 and 20 °C), *L. monocytogenes* growth was higher for pears of more advanced ripeness stages.

Therefore, it is important for companies to have good harvesting techniques and handling and storage practices to prevent pathogen contamination. Additionally, it is important that the fruit product's temperature is maintained at less than 5 °C until consumption to avoid exceeding the microbiological safety criteria of *L. monocytogenes* (EC No 1441/2007).

Acknowledgements

The authors are grateful to the University of Lleida, Grupo Alimentario Citrus, Banco Santander and the Spanish Government (Ministerio de Economia y Competitividad, research project AGL-2012-38671) for its financial support.

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Highlights

- *Listeria monocytogenes* grew on pears at different ripeness stages, even at 5 °C.
- *L. monocytogenes* growth on fresh-cut pears increased with increasing temperature.
- pH, acidity and soluble solids content did not change after conditioning the pears.
- Pear ripeness is not an important factor in preventing *L. monocytogenes'* growth at standard refrigeration temperatures.

Table 1. Physicochemical characteristics of the flesh of whole 'Conference' pears prior to use in experiments at 5 °C.

Fruit ripeness stage	Firmness (N)	pH	Soluble solid content (°Brix)	Titratable acidity (g ac. malic L^{-1})
Mature-green	56.3 ± 3.1 [a]	5.05 ± 0.17[ab]	14.6 ± 0.7[a]	1.26 ± 0.07[b]
Partially ripe	48.1 ± 2.2 [b]	5.17 ± 0.14[a]	14.6 ± 0.8[a]	1.23 ± 0.18[b]
Ripe	37.4 ± 3.3 [c]	5.02 ± 0.22[ab]	14.7 ± 0.8[a]	1.60 ± 0.36[a]
Overripe	20.2 ± 3.9 [d]	4.85 ± 0.28[b]	15.1 ± 1.1[a]	1.37 ± 0.17[ab]

Values are expressed as the mean of six values ± standard deviation. For each parameter, different lowercase letters (a, b, c and d) in the same column indicate significant differences (P< 0.05) according to the LSD test.

Table 2. Physicochemical characteristics of the flesh of whole 'Conference' pears prior to use in experiments at 10 °C.

Fruit ripeness stage	Firmness (N)	pH	Soluble solid content (°Brix)	Titratable acidity (g ac. malic L^{-1})
Mature-green	56.8 ± 3.1[a]	4.70 ± 0.24[b]	15.3 ± 0.2[a]	1.56 ± 0.26 [a]
Partially ripe	48.2 ± 3.2[b]	5.03 ± 0.32[a]	14.9 ± 0.2 [a]	1.24 ± 0.45 [ab]
Ripe	36.1 ± 3.8[c]	5.17 ± 0.28[a]	14.9 ± 0.5 [a]	1.18 ± 0.18 [b]
Overripe	21.2 ± 4.5[d]	4.93 ± 0.19[ab]	14.7 ± 0.7 [a]	1.21 ± 0.27 [b]

Values are expressed as the mean of six values ± standard deviation. For each parameter, different lowercase letters (a, b, c and d) in the same column indicate significant differences (P< 0.05) according to the LSD test.

512 **Table 3.** Physicochemical characteristics of the flesh of whole 'Conference' pears prior to use in
513 experiments at 20 °C.

Fruit ripeness stage	Firmness (N)	pH	Soluble solid content (°Brix)	Titratable acidity (g ac. malic L^{-1})
Mature-green	58.6 ± 3.7[a]	4.99 ± 0.19[a]	14.7 ± 0.7[ab]	1.04 ± 0.07[a]
Partially ripe	50.6 ± 2.6[b]	5.00 ± 0.27[a]	14.9 ± 0.2[ab]	0.84 ± 0.15[b]
Ripe	36.0 ± 2.6[c]	5.04 ± 0.25[a]	15.1 ± 0.5[a]	0.99 ± 0.29[ab]
Overripe	20.6 ± 2.1[d]	4.91 ± 0.14[a]	14.6 ± 0.5[b]	1.05 ± 0.15[a]

514 Values are expressed as the mean of six values ± standard deviation. For each parameter, different
515 lowercase letters (a, b, c and d) in the same column indicate significant differences (P< 0.05) according
516 to the LSD test.

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518

519 **Table 4.** Results obtained from a growth potential test for *L. monocytogenes* in ready-to-eat
520 pears. The values in table were comparable between 5 and 10 °C because both experiments
521 were carried out for 8 days. Moreover, this table compares all growth potential values after 2
522 days of storage at 5, 10 and 20 °C.

523

	After 2 days of storage (log CFU g^{-1})			After 8 days of storage (log CFU g^{-1})	
	5 °C	**10 °C**	**20 °C**	**5 °C**	**10 °C**
Mature-green (54-60 N)	0.42	1.57	4.11	2.41	3.48
Partially ripe (43-53 N)	0.36	1.73	3.84	2.11	3.73
Ripe (31-42 N)	0.33	1.70	4.41	2.44	4.00
Overripe (< 31 N)	0.18	1.77	4.41	2.19	4.40

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Figure captions

Fig. 1 *Listeria monocytogenes* population on fresh-cut 'Conference' pears processed at different ripeness stages stored at 5 °C. The values are the average of triplicate samples from two experiments (n=6). The error bars represent the standard deviation of the mean. Different lowercase letters (a, b, c and d) in the same point indicate significant differences ($P < 0.05$) among firmness states of a pear at each sampling time. There are no letters for points when there were no significant differences.

Fig. 2 *Listeria monocytogenes* population on fresh-cut 'Conference' pears processed at different ripeness stages stored at 10 °C. The values are the average of triplicate samples from two experiments (n=6). The error bars represent the standard deviation of the mean, and different lowercase letters (a, b, c and d) in the same point indicate significant differences ($P < 0.05$) among firmness states of a pear at each sampling time. There are no letters for points when there were no significant differences.

Fig. 3 *Listeria monocytogenes* population on fresh-cut 'Conference' pears processed at different ripeness stages stored at 20 °C. The values are the average of triplicate samples from two experiments (n=6). The error bars represent the standard deviation of the mean, and different lowercase letters (a, b, c and d) in the same point indicate significant differences ($P < 0.05$) among firmness states of a pear at each sampling time. There are no letters for points when there were no significant differences.

Figure 1

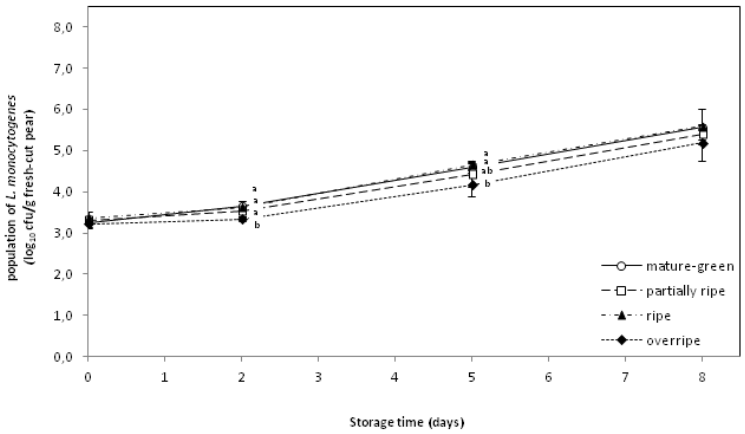

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Figure 2

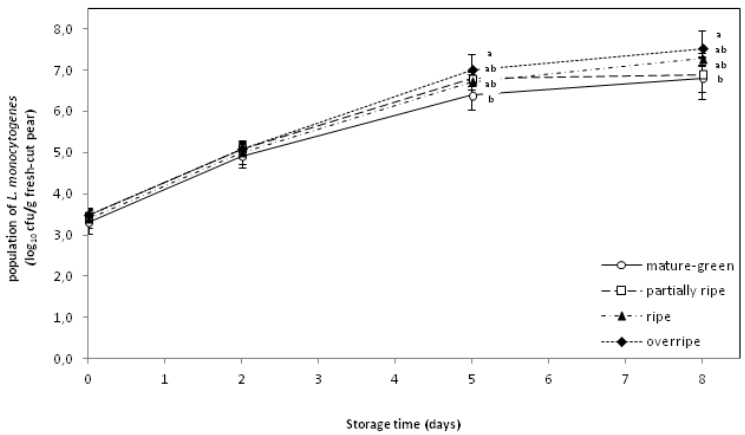

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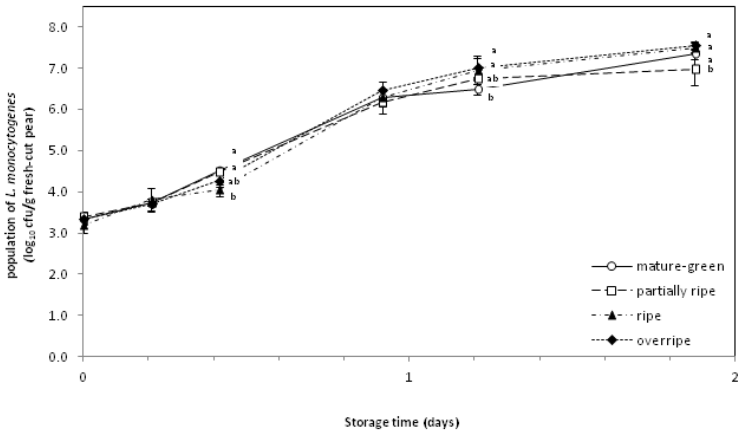
Figure 3



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