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## Highlights

- *L. monocytogenes* was reduced by 1-log unit in presence of *P. graminis* CPA-7
- No effect of CPA-7 was observed against *S. enterica*
- SSC and TA of fresh-cut pears was not negatively affected by CPA-7 nor CaCl<sub>2</sub> treatment
- Ethanol and acetaldehyde increased during shelf-life regardless of CPA-7 presence
- CPA-7 affected the volatile profile of fresh-cut pears



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62 **14 ABSTRACT**  
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64 15 The application of microorganisms to control the growth of foodborne pathogens is an  
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66 16 alternative to the use of chemical additives. In this work, *Pseudomonas graminis* CPA-7  
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68 17 was tested as a biocontrol agent against *Salmonella enterica* and *Listeria*  
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70 18 *monocytogenes* on fresh-cut pear under conditions that simulate its commercial  
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72 19 application at  $5 \pm 1$  °C (under a modified atmosphere and antioxidant solution). The  
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74 20 quality of the fresh-cut fruit, including the ethanol and acetaldehyde contents and the  
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76 21 volatile profile, was determined. After the storage period, the *L. monocytogenes*  
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78 22 population was reduced by 1-log unit by the presence of CPA-7; however, CPA-7 was  
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80 23 not found to have antagonistic activity against *S. enterica*. The fruit quality (total soluble  
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82 24 solids content and titratable acidity) was not negatively affected by CPA-7. The ethanol  
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84 25 and acetaldehyde contents increased during the shelf-life of the fruit regardless of the  
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86 26 presence of CPA-7. Some volatile compounds were key factors for discriminating  
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88 27 samples from the two groups (the control group and the group that was inoculated with  
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90 28 CPA-7). Some components are common in the volatile profile of pear (methyl acetate,  
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92 29 3-methylbutyl acetate, 1-butanol, 1-hexanol, and hexanal), and thus increases in their  
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94 30 contents could enhance consumers flavour perception.

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97 32 **Keywords:** *Listeria*, *Salmonella*, ethanol, acetaldehyde, antagonist  
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121 **34 1. Introduction**  
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123 35 The consumption of fruits and vegetables provides us with a large amount of  
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125 36 micronutrients; therefore, they are basic components of a healthy diet. Many studies  
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127 37 have reported that the intake of fruits and vegetables reduces the risk of mortality due to  
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129 38 cancer and cardiovascular diseases (Wang et al., 2014). Therefore, the production of  
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131 39 fresh-cut fruits and vegetables is increasing because of their health benefits as well as  
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133 40 their convenience for consumers.

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135 41 Minimal fruit and vegetable processing consists of washing, trimming, peeling, cutting or  
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137 42 shredding, sanitizing and packing. However, these operations do not guarantee the total  
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139 43 elimination of spoilage and foodborne pathogenic microorganisms that could be present  
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141 44 in the produce. Several outbreaks associated with the consumption of fresh-cut produce  
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143 45 have been reported in recent years (CDC, 2016). Chemical sanitizers and additives are  
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145 46 used to preserve fresh-cut produce; however, consumer's concerns regarding these  
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147 47 substances in food has promoted the development of alternative techniques.

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150 48 One such method is biopreservation or biological control. Non-pathogenic  
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152 49 microorganisms have been proposed as biocontrol agents. They control the growth of  
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154 50 spoilage and pathogenic microorganisms by competing for nutrients or physical space  
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156 51 or by producing substances that negatively affect pathogens (Parish et al., 2003).  
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158 52 Moreover, some lactic acid bacteria (LAB) have also been studied as biocontrol agents.  
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160 53 For example, *Lactobacillus rhamnosus* GG has been reported to control the growth of  
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162 54 foodborne pathogens on fresh-cut apple (Alegre et al., 2011) and on fresh-cut pear  
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164 55 (Iglesias et al., 2017) and *Lactobacillus plantarum* CIT3 on minimally processed apple  
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166 56 (Siroli et al., 2015b). The native microbiota present in fruits and vegetables have also  
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168 57 shown antagonistic activity against foodborne pathogens. Leverentz et al. (2006)  
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170 58 reported that *Candida* spp., *Discosphaerina fagi*, *Gluconobacter assai* and  
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172 59 *Metschnikowia pulcherrima* controlled *L. monocytogenes* and *Salmonella* growth at 10  
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174 60 and 25 °C on fresh-cut apple. Trias et al. (2008) showed that some *Leuconostoc* strains

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180 61 have bactericidal effects against *L. monocytogenes* and reduced the growth of  
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182 62 *Escherichia coli* and *Salmonella typhimurium* on fresh-cut apple at 25 °C. *Pseudomonas*  
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184 63 *graminis* CPA-7, isolated from the surface of an apple, has shown activity against  
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186 64 foodborne pathogens on fresh-cut apple and peach (Alegre et al., 2013b) and on fresh-  
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188 65 cut apple and melon under conditions simulating commercial applications (Abadias et  
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190 66 al., 2014; Alegre et al., 2013a). Recently, Iglesias et al. (2018) demonstrated that this  
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192 67 biocontrol agent is also effective on fresh-cut pear. Among the many requirements,  
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194 68 biopreservation cultures should not impact the quality of the fresh-cut fruit through  
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196 69 possible metabolic reactions during bacterial growth.

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199 70 Maintaining the sensorial qualities of minimally processed fruit after processing and  
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201 71 during the chain of distribution is very difficult. The shelf-life of cut produce is very limited  
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203 72 due to browning of the flesh and the loss of flavour (Conway et al., 2002; Toivonen, 2006;  
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205 73 Toivonen and Delaquis, 2006). Some factors including variety, ripeness stage, and the  
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207 74 atmosphere and temperature of storage affect shelf-life during postharvest storage  
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209 75 following processing. Modified atmosphere packaging (MAP) in combination with  
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211 76 refrigeration temperatures is used to preserve fresh-cut produce. Low O<sub>2</sub> and high CO<sub>2</sub>  
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213 77 can be used to preserve the quality of minimally processed fruit because they inhibit the  
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215 78 bioreactions in fruit tissue that may lead to physiological decay (Rosen and Kader, 1989;  
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217 79 Sapers and Miller, 1998). However, that gas composition may initiate fermentative  
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219 80 pathways that release metabolites such as ethanol that cause off-flavours (Soliva-  
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221 81 Fortuny et al., 2002). Moreover, it is known that although a high CO<sub>2</sub> level can inhibit  
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223 82 aerobic spoilage microorganisms, it can also allow pathogen growth (Rodriguez-Aguilera  
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225 83 et al., 2009). Therefore, it is necessary to maintain an O<sub>2</sub> concentration that is sufficiently  
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227 84 low but also over the fermentation threshold (Lakakul et al., 1999).

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229 85 Concerning firmness, postharvest calcium dips for whole fruit have been demonstrated  
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231 86 to preserve firmness, cell wall structure (Glenn and Poovaiah, 1990), nutritional quality  
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233 87 (Goldberg, 1984) and fruit flavour (Ortiz et al., 2009). Similarly, combinations of calcium

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239 88 treatment (0.5-4 %) with packaging under modified atmospheres and low storage  
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241 89 temperature ( $< 5\text{ }^{\circ}\text{C}$ ) are generally effective for extending the shelf-lives of minimally  
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243 90 processed products.

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246 91 The aim of this study was to evaluate the antagonistic effect of CPA-7 against *Salmonella*  
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248 92 and *L. monocytogenes* on fresh-cut pear treated with  $\text{CaCl}_2$  after harvest under  
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250 93 conditions simulating commercial applications (under MAP and in presence of an  
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252 94 antioxidant solution) at  $5 \pm 1\text{ }^{\circ}\text{C}$ . In addition, the effect of CPA-7 on some quality  
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254 95 parameters, including ethanol and acetaldehyde contents and the volatile profile, were  
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256 96 evaluated throughout storage.

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298 **99 2. Materials and Methods**  
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300 2.1. Bacterial strains and inoculum preparation  
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302  
303 101 As pathogen microorganisms, five serovars of *Salmonella enterica* subsp. *enterica* were  
304 102 used, namely, Agona (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC  
305 103 BAA-710), Gaminara (ATCC BAA-711) and Enteritidis (CECT-4300), along with five  
306 104 serovars of *Listeria monocytogenes*, namely, serovar 1a (CECT 4031), serovar 3a  
307 105 (CECT 933); serovar 4d (CECT 940), serovar 4b (CECT 4032) and serovar 1/2a, which  
308 106 had previously been isolated in our laboratory from a fresh-cut lettuce sample (Abadias  
309 107 et al., 2008). *S. enterica* and *L. monocytogenes* strains were grown individually in  
310 108 tryptone soy broth (TSB, Biokar Diagnostics, France) medium and in TSB supplemented  
311 109 with 6 g L<sup>-1</sup> of yeast extract (TSBYE), respectively, for 20-24 h at 37 ± 1 °C.  
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321 110 *Pseudomonas graminis* strain CPA-7 (deposit number CBS 136973, Centraalbureau  
322 111 voor Schimmelcultures, CBS, Utrech, The Netherlands), isolated in our lab from the  
323 112 surface of an apple (Alegre et al., 2013b), was used as antagonist. It was grown in TSB  
324 113 for 20-24 h at 25 ± 1 °C. Bacterial cells were harvested by centrifugation at 9800 x g for  
325 114 10 min at 10 °C. Afterwards, the pathogen cells were resuspended in saline solution (SS;  
326 115 8.5 g L<sup>-1</sup> NaCl), and the CPA-7 cells were suspended in sterile distilled water. A single  
327 116 suspension of the five *S. enterica* serovars and the *L. monocytogenes* serovars was  
328 117 produced by mixing equal volumes of each concentrated suspension.  
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337 118 To inoculate the fruit, an aliquot of each of the concentrated bacterial suspensions was  
338 119 added to an antioxidant solution (2 % ascorbic acid + 2 % sodium citrate + 1 % CaCl<sub>2</sub>),  
339 120 which was selected based on previous studies (Iglesias et al., 2018), to obtain solutions  
340 121 of approximately 10<sup>5</sup> cfu mL<sup>-1</sup> in the case of *S. enterica* and *L. monocytogenes* and  
341 122 10<sup>7</sup> cfu mL<sup>-1</sup> for CPA-7. Inoculum concentrations were checked by plating appropriate  
342 123 dilutions onto XLD (xylose-lysine-deoxycholate Agar, Biokar Diagnostics, France) for  
343 124 *S. enterica*, onto Palcam agar (Palcam Agar Base with selective supplementation, Biokar  
344 125 Diagnostics, France) for *L. monocytogenes*, and onto tryptone soy agar (TSA, Biokar  
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357 126 Diagnostics, France) for CPA-7. The plates were incubated at  $37 \pm 1$  °C for 24 for  
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359 127 *S. enterica*, at  $37 \pm 1$  °C 48 h for *L. monocytogenes*, and at  $30 \pm 1$  °C for 48 h for CPA-  
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## 363 364 129 2.2. Fruit processing

365  
366 130 'Conference' pears (*Pyrus communis* L. cv. Conference) were used in this study. After  
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368 131 harvest, the pears were divided in two lots. Whole fruits of lot 1 were dipped in water at  
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370 132 25 °C for 5 min (control group), and whole fruits of lot 2 were dipped in a solution  
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372 133 containing 10 g L<sup>-1</sup> CaCl<sub>2</sub> at 25 °C for 5 min. Afterwards, the pears of both lots were  
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374 134 stored at  $0 \pm 1$  °C for 5 months in a controlled atmosphere (2 kPa O<sub>2</sub> and 1 kPa CO<sub>2</sub>)  
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376 135 leading up to the experiment.  
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378 136 After this storage period, the pears were stored at 20 °C until they reached the optimum  
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380 137 ripeness stage for processing ( $44 \pm 3.2$  N) (Soliva-Fortuny et al., 2004). Prior to the  
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382 138 experimental studies, the pears were sanitized by immersion into a 0.1 g L<sup>-1</sup> NaOCl  
383  
384 139 solution adjusted to pH 6.5 using citric acid and then rinsed and dried. After that, the  
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386 140 pears were peeled and cut into 10 wedges using a handheld apple corer and slicer.  
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## 388 389 141 2.3. Fruit inoculation and packaging

390  
391 142 To carry out the experiment, the following treatments were prepared: (a) control:  
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393 143 antioxidant solution; (b) Sal + Lm: antioxidant solution inoculated with *S. enterica* and  
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395 144 *L. monocytogenes* at 10<sup>5</sup> cfu mL<sup>-1</sup>; (c) CPA-7: antioxidant solution with 10<sup>7</sup> cfu mL<sup>-1</sup> CPA-  
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397 145 7 cells; and (d) Sal + Lm + CPA-7: antioxidant solution containing *S. enterica* and  
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399 146 *L. monocytogenes* (10<sup>5</sup> cfu mL<sup>-1</sup>) and CPA-7 (10<sup>7</sup> cfu mL<sup>-1</sup>). The pear wedges were  
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401 147 dipped into these solutions (1:2 w/v) for 2 min in an orbital shaker at 150 rpm on an orbital  
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403 148 shaker. After that, the fresh-cut pears were allowed to dry open to air at room  
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405 149 temperature. Approximately  $120 \pm 5$  g of pear wedges were placed in 400-mL  
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407 150 polyethylene terephthalate ShelfMaster™ Pronto™ trays (PlusPack, Denmark) and  
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409 151 sealed with peelable plastic with an O<sub>2</sub> permeability of 180 cm<sup>3</sup> m<sup>-2</sup> day<sup>-1</sup> atm<sup>-1</sup> at 23 °C  
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416 152 (film PET OLAF interior and OPP exterior with a line of holes of 60 - 80 µm each and 75  
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418 153 mm apart from each other). The film used in this study was selected based on the results  
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420 154 of previous studies according to the quality parameters of the fresh-cut pear and the  
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422 155 survival and efficacy of CPA-7 (Iglesias et al., 2018).

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425 156 The trays of fresh-cut pears were stored at  $5 \pm 1$  °C. Microorganism populations were  
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427 157 determined the day of inoculation and after 2, 6 and 9 days of storage in the three sample  
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429 158 trays. The *S. enterica* and *L. monocytogenes* populations were evaluated in treatments  
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431 159 (b) and (d), and CPA-7 was evaluated in treatment (c). Total aerobic mesophilic counts  
432  
433 160 (TAM) were determined in control samples (a). For analysis, 10 g of pear from each tray  
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435 161 was mixed with 90 mL of buffered peptone water (BPW, Oxoid, LTD, Basingstoke,  
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437 162 Hampshire, England) in a sterile bag and homogenized in a masticator (IUL Instruments,  
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439 163 Barcelona, Spain) set at 8.5 strokes s<sup>-1</sup> for 90 s. Serial dilutions were prepared with saline  
440  
441 164 peptone (SP; 8.5 g L<sup>-1</sup> NaCl and 1 g L<sup>-1</sup> peptone), and the solutions were plated in  
442  
443 165 duplicate onto Palcam (*L. monocytogenes*), XLD (*S. enterica*) and on Plate Count Agar  
444  
445 166 (Biokar Diagnostics, France). The agar plates were incubated at  $37 \pm 1$  °C for 24 h for  
446  
447 167 *S. enterica*, at  $37 \pm 1$  °C for 48 h for *L. monocytogenes*, at  $30 \pm 1$  °C for 48 h for CPA-7  
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449 168 and at  $30 \pm 1$  °C for 72 h for TAM.

## 450 451 169 2.5. Determination of the physical and chemical parameters

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454 170 To determine if the presence of CPA-7 impacts the quality of the fresh-cut pear, quality  
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456 171 parameters were measured in treatments (a) and (c) (without foodborne pathogens).  
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458 172 Three determinations (one per each tray) per treatment were made.

### 459 460 173 2.5.1. Headspace gas composition

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463 174 Before each microbial analysis at each sampling time, the O<sub>2</sub> and CO<sub>2</sub> concentrations  
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465 175 inside the trays were measured using a handheld gas analyser (CheckPoint O<sub>2</sub>/CO<sub>2</sub>, PBI  
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467 176 Dansensor, Denmark). An adhesive septum was attached to the film, and a needle was  
468  
469 177 used to determine the gas composition. The results are expressed as kPa.

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475 178 2.5.2. Measurement of soluble solids content and titratable acidity  
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478 179 At each sampling time, the soluble solids content (SSC) in juice extracted by crushing  
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480 180 the pear wedges in a blender was measured at 20 °C with a handheld refractometer  
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482 181 (Atago Co. Ltd., Tokyo, Japan). The results are expressed as %.

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485 182 To measure the titratable acidity (TA), three measurements per treatment were made at  
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487 183 each sampling point. Ten millilitres of pear juice was diluted with 10 mL of distilled water,  
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489 184 and the solution was titrated with 0.1 N NaOH up to pH 8.2. The results were calculated  
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491 185 as g of malic acid per litre of solution.

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493 186 2.5.3. Ethanol and acetaldehyde headspace concentrations  
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496 187 The contents of ethanol and acetaldehyde were determined according to the protocol  
497  
498 188 described by Echeverría et al. (2004) with slight modifications. These compounds were  
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500 189 extracted from the same juice used to determine SSC and TA. Juice samples (5 mL)  
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502 190 were stored at -20 °C until analysis. Samples were transferred to a 10-mL test tube with  
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504 191 a screw cap and incubated in a water bath at 60 °C. After 60 min, a 1 mL samples of the  
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506 192 headspace gas was taken with a syringe and injected into an Agilent Technologies  
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508 193 6890N gas chromatograph (GC) for the determination of both the acetaldehyde and  
509  
510 194 ethanol concentrations. To do this, the gas chromatograph was equipped with a flame  
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512 195 ionisation detector (FID) and a column (2 m × 2 mm i.d.) containing 5 % Carbowax on  
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514 196 60/80 Carbopack (Supelco, Bellefonte, PA, USA). The temperature of the injector,  
515  
516 197 detector and oven were 180, 220 and 80 °C, respectively. Tissue concentrations were  
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518 198 calculated using ethanol and acetaldehyde calibration curves prepared by measuring the  
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520 199 headspace of Milli-Q water spiked with a known amount of ethanol and acetaldehyde at  
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522 200 increasing concentrations and are expressed as  $\mu\text{L L}^{-1}$ .

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524 201 2.5.4. Determination of the volatile compounds  
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534 202 Headspace solid-phase microextraction (HS-SPME) was used for the extraction and to  
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536 203 determine the concentrations of the volatile compounds. SPME fibres coated with a 65-  
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538 204  $\mu\text{m}$  layer of polydimethylsiloxane–divinylbenzene (65  $\mu\text{m}$  PDMS/DVB; Supelco Co.,  
539  
540 205 Bellefonte, PA, USA) were used. Fibres were activated before sampling according to the  
541  
542 206 manufacturer’s instructions.

544  
545 207 Four pieces of fruit per tray ( $n = 3$ ) for each treatment were cut in small pieces, frozen  
546  
547 208 with liquid  $\text{N}_2$ , crushed, and immediately transferred to  $-80\text{ }^\circ\text{C}$  storage until the volatile  
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549 209 components could be analysed.

551 210 For each extraction, 4 g of the homogenized crushed pulp was placed into a 20-mL  
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553 211 screw-cap vial containing 0.5 g of NaCl to facilitate the release of volatile compounds.  
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555 212 Prior to sealing the vial, 1  $\mu\text{L}$  of  $0.086\text{ mg L}^{-1}$  butyl benzene/diethyl ether was added as  
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557 213 an internal standard, and the solution was mixed with a glass rod. A magnetic stirrer was  
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559 214 added to each vial, and the vials were placed into a constant-temperature water bath at  
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561 215  $60\text{ }^\circ\text{C}$  with stirring. Samples were equilibrated for 20 min, and then the SPME fibres were  
562  
563 216 exposed to the head space of the sample for 30 min to adsorb the analytes according to  
564  
565 217 the procedure described by Qin et al. (2012). The volatile compounds were subsequently  
566  
567 218 desorbed over 10 min at  $240\text{ }^\circ\text{C}$  into the splitless injection port of the chromatograph.  
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569 219 The volatile constituents were identified and quantified with an HP 5890A gas  
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571 220 chromatograph with a flame ionization detector equipped with a capillary column with  
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573 221 cross-linked free fatty acids as the stationary phase (FFAP;  $50\text{ m} \times 0.2\text{ mm} \times 0.33\text{ }\mu\text{m}$ ).  
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575 222 Helium was used as the carrier gas at a constant flow of  $1.0\text{ mL min}^{-1}$ . The injector and  
576  
577 223 detector temperatures were  $240\text{ }^\circ\text{C}$ . The oven temperature programme was  $40\text{ }^\circ\text{C}$  for 1  
578  
579 224 min, increasing at  $2.5\text{ }^\circ\text{C min}^{-1}$  to  $115\text{ }^\circ\text{C}$ , then increasing at  $8\text{ }^\circ\text{C min}^{-1}$  to  $225\text{ }^\circ\text{C}$  and  
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581 225 holding for 15 min. Compounds were identified by comparing their respective retention  
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583 226 index with those of standards. All of the standards for the volatile compounds studied in  
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585 227 this work were analytical grade or the highest quality available. Quantification was  
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593 228 performed using individual calibration curves for each identified compound. The  
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595 229 concentrations of volatile compounds were expressed as ng g<sup>-1</sup>.  
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597 230 Compound identification was performed on an Agilent 6890N gas chromatograph/mass  
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599 231 spectrometer (Agilent Technologies, Inc.) using the same capillary column as was used  
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601 232 in the GC analyses. Mass spectra were obtained by electron impact ionization at 70 eV.  
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603 233 Helium was used as the carrier gas, and the same temperature gradient programme  
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605 234 described previously was used for MS acquisition. Spectrometric data were recorded  
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607 235 (Hewlett-Packard 3398 GC Chemstation) and compared with those from the original  
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609 236 NIST HP59943C library mass spectra.  
610

## 611 237 2.6. Statistical analysis

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614 238 Prior to ANOVA, cfu g<sup>-1</sup> data were converted to log<sub>10</sub> cfu g<sup>-1</sup>. Other data were not  
615  
616 239 converted. Data were analysed using general linear model analysis with JMP®8 software  
617  
618 240 (JMP®8, SAS Institute, Cary, NC, USA). After analysis of variance (ANOVA), significant  
619  
620 241 differences between treatments for each sampling time were analysed by Student's t test  
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622 242 or Tukey's test at a significance level of P < 0.05.  
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624  
625 243 Unscrambler version 9.1.2. Software (CAMO, 2004) was used to develop a partial least  
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627 244 square regression (PLSR) model. The PLSR model was used as a predictive method to  
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629 245 relate the CPA-7 population (Y) to a set of explanatory variables (X), which include the  
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631 246 volatile compound emissions and O<sub>2</sub> and CO<sub>2</sub> concentrations. As a pretreatment, the  
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633 247 data were centred and weighted using the inverse of the standard deviation of each  
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635 248 variable in order to avoid the influence of the different scales used for the variables  
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637 249 (Martens and Naes, 1989). A full cross validation was run as a validation procedure.  
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652 **3. Results**  
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655 251 3.1. Population of microorganisms on fresh-cut pear stored at 5 °C  
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658 252 Initial *S. enterica* populations (Fig. 1A) were approximately 3.40 log cfu g<sup>-1</sup> regardless  
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660 253 CaCl<sub>2</sub> treatment and the presence of CPA-7. The *S. enterica* population decreased  
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662 254 throughout the storage time (9 days) by more than 0.5-log units, and significant  
663  
664 255 differences were observed between the initial and final values. Neither CPA-7 nor CaCl<sub>2</sub>  
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666 256 postharvest treatment were found to have an effect against *S. enterica* under the  
667  
668 257 conditions tested.

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670 258 The initial populations of *L. monocytogenes* were between 2.80 and 3.00-log units after  
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672 259 the inoculation of the pear wedges (Fig. 1B). When pears treated with CPA-7 but  
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674 260 untreated or treated with CaCl<sub>2</sub> were compared, significant differences in the population  
675  
676 261 were reported after 2 and 6 days of storage for both treatments (b and d). The  
677  
678 262 populations of *L. monocytogenes* on fresh-cut pear and pear untreated with CPA-7  
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680 263 increased during the storage time and reached similar values (5.62 ± 0.11 log cfu g<sup>-1</sup> on  
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682 264 CaCl<sub>2</sub>-treated pear and 5.65 ± 0.15 log cfu g<sup>-1</sup> on CaCl<sub>2</sub>-untreated pear wedges). On  
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684 265 pear wedges treated with CPA-7, the final *L. monocytogenes* population was not  
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686 266 influenced by the CaCl<sub>2</sub> treatment and reached values of 4.71 ± 0.22 log cfu g<sup>-1</sup> on pear  
687  
688 267 wedges treated with CaCl<sub>2</sub> and 4.88 ± 0.21 log cfu g<sup>-1</sup> on untreated pear wedges. CPA-  
689  
690 268 7 significantly reduced (approximately 1-log unit) the population of *L. monocytogenes*  
691  
692 269 after 9 days of storage at 5 ± 1 °C.

693  
694 270 Regardless of the postharvest CaCl<sub>2</sub> treatment, initial CPA-7 populations (treatment c)  
695  
696 271 (Fig. 1C) were the same (5.59 ± 0.06 and 5.54 ± 0.06 log cfu g<sup>-1</sup> on pear wedges  
697  
698 272 untreated and treated with CaCl<sub>2</sub>, respectively). Both populations increased after 9 days  
699  
700 273 of storage and reached 6.61 ± 0.03 and 7.09 ± 0.05 log cfu g<sup>-1</sup> on pear wedges untreated  
701  
702 274 and treated with CaCl<sub>2</sub>, respectively. Populations on pear wedges treated with CaCl<sub>2</sub>  
703  
704 275 increased faster than populations on fresh-cut pear not treated with CaCl<sub>2</sub>. Significant  
705  
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711 276 differences were found at 2 and 9 days of storage. Regardless of the CaCl<sub>2</sub> postharvest  
712  
713 277 treatment, the population of TAM in pear wedges not treated with CPA-7 did not exceed  
714  
715 278 3.50 log cfu g<sup>-1</sup> during the experiment (data not shown).  
716  
717

### 718 279 3.2. Headspace gas concentration

720  
721 280 The O<sub>2</sub> concentration decreased from 21.0 kPa to between 12.6 and 14.6 kPa after 9  
722  
723 281 days of storage (Table 1), and there were no significant differences from the treatments  
724  
725 282 at any of the tested times. The CO<sub>2</sub> concentration increased throughout the storage  
726  
727 283 period until it reached values from 7.8-9.7 kPa. Except at day 2, no significant effects of  
728  
729 284 CaCl<sub>2</sub> and CPA-7 treatments were found.

### 730 731 285 3.3. Soluble solids content (SSC) and titratable acidity (TA)

732  
733  
734 286 The SSC ranged from 13.0 to 14.8 % during the assay (Table 2). The SSC values of  
735  
736 287 postharvest CaCl<sub>2</sub>-treated (CaControl and CaCPA-7) pears were higher than those of  
737  
738 288 the CPA-7-treated pears. In general, the SSCs were also significantly lower for CPA-7-  
739  
740 289 treated fresh-cut pears.

741  
742 290 There were not significant differences in the TA prior to the different treatments (Table  
743  
744 291 2). After 2, 6 and 9 days of storage, the TA values of the CaCl<sub>2</sub>-untreated pear samples  
745  
746 292 (Control and CPA-7) were similar. There were no significant differences in the TA due to  
747  
748 293 the presence of CPA-7 in the CaCl<sub>2</sub>-treated pears (CaControl and CaCPA-7). The TA  
749  
750 294 was only influenced by CPA-7 after 9 days of storage in CaCl<sub>2</sub>-untreated pears; the TA  
751  
752 295 value was significantly lower (1.27 g L<sup>-1</sup>) for fresh-cut pears treated with CPA-7 than for  
753  
754 296 CPA-7-untreated ones (1.70 g L<sup>-1</sup>). At the end of the storage period, each TA value was  
755  
756 297 significantly lower than the initial value for all treatments.

### 757 758 759 298 3.4. Ethanol and acetaldehyde concentrations

760  
761 299 The initial concentrations of ethanol were between 40.8 and 70.6 mL L<sup>-1</sup>, and no  
762  
763 300 significant differences were observed between treatments (Fig. 2A). After 6 days of  
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765  
766  
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768  
769  
770 301 storage, the ethanol concentration was significantly higher in the CaCl<sub>2</sub>-treated pear  
771  
772 302 samples without CPA-7 (Ca Control, 175.2 mL L<sup>-1</sup>) than in other treatments. The ethanol  
773  
774 303 concentrations in the fresh-cut pears significantly increased (by a factor of approximately  
775  
776 304 two) during the storage period in all treatments regardless of the presence of CPA-7.  
777  
778 305 Thus, the increase could not be attributed to the biopreservation culture.

780  
781 306 The initial concentration of acetaldehyde was between 3.1 and 4.3 mL L<sup>-1</sup> (Fig. 2B). The  
782  
783 307 acetaldehyde concentration increased throughout storage, reached its maximum levels  
784  
785 308 after 6 days, and then remained constant. No significant differences were observed  
786  
787 309 between treatments at the end of the storage.

### 788 789 310 3.5. Volatile compound emissions

791  
792 311 Tables 3 and 4 show the mean concentrations of the volatile compounds emitted by the  
793  
794 312 pear wedges on the day of the assay (0 days) and after 2 and 6 days of storage at 5 ±  
795  
796 313 1 °C. A total of 43 compounds (25 esters, 10 alcohols, 4 aldehydes, 1 terpene, 2 ketones  
797  
798 314 and 1 acid) were identified and quantified in the volatile fraction emitted by minimally  
799  
800 315 processed fruit. Differences in the volatile profiles were found both before and after cold  
801  
802 316 storage as a function of the postharvest CaCl<sub>2</sub> treatment. Two esters (hexyl butanoate  
803  
804 317 and hexyl 2-methylbutanoate) and one ketone (6-methyl-5-hepten-2-one) were not  
805  
806 318 detected in the volatile profile of the pears treated with calcium chloride (Table 3).

807  
808 319 In pears treated with CaCl<sub>2</sub> after harvest (Table 3), the storage period and inoculation  
809  
810 320 with CPA-7 influenced the contents of individual volatile compounds. Thus, butyl 2-  
811  
812 321 methylbutanoate and ethyl hexanoate were detected for the first time after 6 days at 5  
813  
814 322 °C.

815  
816  
817 323 Different results were obtained in pears untreated with CaCl<sub>2</sub> (Table 4); four esters (ethyl  
818  
819 324 2-methylbutanoate, 2-methylbutyl-2-methylbutanoate, pentyl-2-methylbutanoate, and  
820  
821 325 ethyl hexanoate) were detected for the first time after 2 days at 5 °C, and 1-pentanol was  
822  
823 326 quantified for the first time after 6 days in CPA-7-inoculated samples.



827  
828  
829 327 Throughout the cold storage period, the effects of inoculation with CPA-7 on the volatiles  
830  
831 328 profile was more important in pears that had not been treated with calcium chloride after  
832  
833 329 harvest. Thus, in this case, minimally processed pears showed higher concentrations of  
834  
835 330 16 volatile compounds (8 esters, 4 alcohols, 3 aldehydes and one terpene) than samples  
836  
837 331 not treated with CPA-7 (Table 4). In contrast, in pear wedges treated with CaCl<sub>2</sub> and  
838  
839 332 inoculated with CPA-7 (Table 3) only 3 esters and 1 alcohol (3-methyl -2-butanol)  
840  
841 333 increased significantly after 2 and 6 days at 5 °C.

842  
843 334 After 6 days of storage at 5 °C, the CaCl<sub>2</sub>-treated pear wedges inoculated with CPA-7  
844  
845 335 showed higher concentrations in 6 of the 43 volatile compounds (16 %) in contrast to the  
846  
847 336 32 volatile compounds that showed higher concentration (74 %) in the non-inoculated  
848  
849 337 CPA-7 samples (Table 3). This difference was mainly due to lower concentrations of  
850  
851 338 aliphatic esters (except methyl and ethyl acetates, ethyl 2-methylbutanoate and butyl  
852  
853 339 butanoate), alcohols (except 3-methyl-2-butanol), aldehydes, α-farnesene and acetic  
854  
855 340 acid than in the samples not treated with CPA-7. Instead, after 6 days of cold storage at  
856  
857 341 5°C, the CaCl<sub>2</sub>-untreated and CPA-7-treated minimally processed pear samples emitted  
858  
859 342 higher amounts of 51 % of the volatile compounds in comparison to 19 % of the  
860  
861 343 compounds in samples not inoculated with CPA-7 (Table 4). This result was due to  
862  
863 344 higher ester concentrations (except ethyl, butyl and hexyl acetates, ethyl 2-  
864  
865 345 methylbutanoate and pentyl 3-methylbutanoate), alcohols (except ethanol, 3-methyl-2-  
866  
867 346 butanol and 1-pentanol), aldehydes (except acetaldehyde), α-farnesene and acetic acid  
868  
869 347 of CPA-7 samples in comparison to the pear wedges not treated with CPA-7.

870  
871 348 A partial least square regression (PLSR) model was developed to evaluate possible  
872  
873 349 correlations between the CPA-7 population (*Y variable*) and a set of potentially  
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875 350 explanatory variables (*X variables*), which included the concentration of the volatile  
876  
877 351 compounds emitted by pear wedges. Samples from day 0 were excluded of this model  
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879 352 to refine the differentiation between the control and pear wedges treated with CPA-7. To  
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881 353 carry out the analysis, all samples were included (those treated (Ca) or untreated with  
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888 354 CaCl<sub>2</sub> (CK) and the samples with CPA-7 (CPA7) or without CPA-7 (Control), stored at 5  
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890 355 ± 1 °C for 2 and 6 days). Therefore, a PLSR analysis including 8 samples and 43 volatile  
891  
892 356 compounds was performed (Fig. 3). According to this model, up to 98 % of the variability  
893  
894 357 was explained by the emission of volatile compounds. The analysis showed two groups;  
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896 358 samples treated with CPA-7 were located on the right side of PC1, which explained 95  
897  
898 359 % of the total variance, and samples without CPA-7 were located on the left side of PC1  
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900 360 (Fig. 3A). The corresponding loadings plot (Fig. 3B) showed that the samples treated  
901  
902 361 with CPA-7 were associated with high concentrations of 1-hexanol and (Z)-2-hexenyl  
903  
904 362 acetate. There was not a clear influence of the volatile compounds on the differentiation  
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906 363 of pear wedges treated or untreated with CaCl<sub>2</sub> after harvest.

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908  
909 364 Fig. 4 shows the regression coefficients for the CPA-7 population vs. the emission of  
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911 365 volatile compounds. This figure allowed us to identify those volatile components that  
912  
913 366 were most influenced by the CPA-7 population. The application of CPA-7 was related to  
914  
915 367 the emissions of six esters (methyl acetate, 3-methylbutyl acetate, (Z)-2-hexenyl acetate,  
916  
917 368 2-methylpropyl butanoate, pentyl acetate, and butyl hexanoate), five alcohols (3-methyl-  
918  
919 369 2-butanol, 1-butanol, 2-methyl-1-butanol, 1-hexanol and (E)-2-hexen-1-ol), one aldehyde  
920  
921 370 (hexanal), and acetone.

922  
923 371

#### 924 925 372 **4. Discussion**

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928 373 In previous studies (Iglesias et al., 2018), we demonstrated that CPA-7 was effective  
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930 374 against *S. enterica* and *L. monocytogenes* on pear wedges at air temperatures of 20, 10  
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932 375 and 5 ± 1 °C and determined the antioxidant solution and film best used for commercial  
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934 376 applications. In this work, we have focused on the antagonistic activity of CPA-7 against  
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936 377 foodborne pathogens under conditions that simulate commercial applications and how  
937  
938 378 the presence of CPA-7 and the CaCl<sub>2</sub> postharvest treatment influences several pear  
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940 379 quality parameters, including the contents of several volatile compounds.

945  
946  
947 380 After harvest of the fruit, cold storage and a controlled atmosphere are essential for  
948  
949 381 delaying the ripening process. Moreover, postharvest dipping in  $\text{CaCl}_2$  prior to storage  
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951 382 extends the commercial life for both whole and minimally processed fruit (Ortiz et al.,  
952  
953 383 2009; Trentham et al, 2008). Calcium can penetrate fruit flesh through lenticels, but  
954  
955 384 cracks in the cuticle play a significant role in calcium entrance into the fruit (Conway et  
956  
957 385 al., 2002; Ortiz et al., 2009). In general,  $\text{CaCl}_2$  treatment after harvest did not improve  
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959 386 CPA-7 effectiveness against foodborne pathogens evaluated; nevertheless, the CPA-7  
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961 387 population was higher on pear wedges treated with  $\text{CaCl}_2$  after harvest than it was on  
962  
963 388 untreated samples. Microorganisms need calcium for their development, survival and  
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965 389 physiological processes (Corbin et al., 2008). Tiwari et al. (1992) observed that an  
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967 390 increase in extracellular  $\text{Ca}^{2+}$  caused an increase in the growth rate of *Rhizobium*  
968  
969 391 *meliloti*. In addition, Onoda et al. (2000) demonstrated that in absence of  $\text{Ca}^{2+}$ , *E. coli*  
970  
971 392 stopped growing and cells became unusual in form and could lyse and die. However, it  
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973 393 has been demonstrated that the amount of calcium required for bacteria depends on the  
974  
975 394 growth conditions (Youatt, 1993).

976  
977 395 CPA-7 was not observed to have antagonistic activity against *S. enterica* under MAP at  
978  
979 396  $5 \pm 1$  °C, and no pathogen growth was observed. Similarly, Alegre et al. (2013a) did not  
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981 397 observe an antagonistic effect against *Salmonella* on apple wedges. Regarding  
982  
983 398 *L. monocytogenes*, we observed an antagonistic effect from CPA-7 after 9 days of  
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985 399 storage at  $5 \pm 1$  °C, and it caused reductions of approximately 1-log unit. Alegre et al.  
986  
987 400 (2013a) also demonstrated an antagonistic effect of CPA-7 against *L. monocytogenes*  
988  
989 401 on apple wedges; however, the effect was greater under air conditions than under MAP;  
990  
991 402 a similar effect was observed by Abadias et al. (2014) for fresh-cut melon. According the  
992  
993 403 review by Siroli et al. (2015a) some biocontrol agents were also able to control spoilage  
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995 404 microorganisms naturally present in minimally processed fruits and vegetables. In our  
996  
997 405 work, the effect of CPA-7 on the spoilage microorganisms was not evaluated. No visible  
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1006 406 symptoms of microbial spoilage were observed neither in CPA-7 and control fresh-cut  
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1008 407 pears during the shelf-life (9 days at 5° C), so we could not reach to a clear conclusion.

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1010  
1011 408 We observed significant differences in the SSC values of untreated and CPA-7-treated  
1012  
1013 409 fresh-cut pear regardless of postharvest CaCl<sub>2</sub> treatment, except after 6 days of storage  
1014  
1015 410 in the case of the CaCl<sub>2</sub>-treated pear wedges. The SSC values of pear wedges treated  
1016  
1017 411 with CPA-7 were 1 % lower than those of untreated pear, which could be perceived by  
1018  
1019 412 the consumers as a less sweet taste. Regarding the TA values, significant differences  
1020  
1021 413 were observed after 6 and 9 days of storage between the CaCl<sub>2</sub>-treated pear inoculated  
1022  
1023 414 with CPA-7 and non-inoculated samples. It is known that consumers can perceive  
1024  
1025 415 differences in the TA if the variation is higher than 0.08 % (Harker et al., 2002). In our  
1026  
1027 416 case, the differences found after 6 and 9 days of storage were lower than this value and  
1028  
1029 417 therefore could not be perceived by consumers. Alegre et al. (2013a) and Abadias et al.  
1030  
1031 418 (2014) did not report significant differences in SSC or TA values among fruit (apple  
1032  
1033 419 wedges or fresh-cut melon) untreated and treated with CPA-7.

1034  
1035 420 The results showed that ethanol and acetaldehyde production was not affected by the  
1036  
1037 421 presence of CPA-7. We observed that the concentration of ethanol increased throughout  
1038  
1039 422 the assay up to 95-179 mL L<sup>-1</sup> regardless of the treatment. The acetaldehyde  
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1041 423 concentration reached its highest values after 6-9 days of storage. The fact that both  
1042  
1043 424 metabolites increased during the storage time regardless of the treatment could indicate  
1044  
1045 425 that the microorganism did not affect to the biosynthesis of these compounds, and they  
1046  
1047 426 were produced by the fruit metabolism.

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1049 427 The volatile profile emitted by minimally processed Conference pear stored at 5 °C was  
1050  
1051 428 determined; esters accounted for more than 57 % of the volatile fraction of Conference  
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1053 429 wedges both treated and untreated with CaCl<sub>2</sub>. Esters are known as the most abundant  
1054  
1055 430 class of compounds observed when using headspace analysis, and they are the volatile  
1056  
1057 431 compounds that contribute the most to the aroma of intact and fresh-cut pears (Chen et

1063  
1064  
1065 432 al., 2006, Bai et al., 2009). The major esters of Conference pear aroma (butyl and hexyl  
1066 433 acetates) are predominant in other intact *Pyrus communis* pears including Comice  
1067 434 (López et al., 2001), d'Anjou (Argenta et al., 2003), and Barlett (Zlatic et al., 2016), and  
1070 435 the esters are highly correlated with the fruity and characteristic pear aroma (Makkumrai  
1071 436 et al., 2014).

1074  
1075 437 The impact of CPA-7 inoculation on the volatile profiles of fresh-cut Conference pears  
1076 438 differed depending on the CaCl<sub>2</sub> treatment and cold storage time. According to the  
1077 439 evaluation of volatile emissions during cold storage, 3 esters and 2 alcohols were only  
1080 440 detected in Conference wedges inoculated with CPA-7 and not treated with CaCl<sub>2</sub>,  
1081 441 namely, 3-methylbutyl acetate, butyl hexanoate, butyl propanoate, 1-hexanol and 1-  
1082 442 octanol. Previous works have shown that 3-methylbutyl acetate and 1-octanol are  
1083 443 present in the volatile emission profiles of intact Comice pears (Makkumrai et al., 2014),  
1084 444 butyl hexanoate is present in d'Anjou pears, and butyl propanoate and 1-hexanol are  
1085 445 present in intact Conference pears (Rizzolo et al., 2005).

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1090  
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1093 446 When the data were analysed using a partial least square regression (PLSR) model, we  
1094 447 could detect 13 volatile compounds (6 were esters, 5 alcohols, 1 aldehyde and 1 ketone)  
1095 448 that were key variables for discriminating the samples in two groups (the control and  
1096 449 inoculated with CPA-7 samples). The key compounds were methyl acetate (fruity, ripe,  
1100 450 and floral notes), 3-methylbutyl acetate (fruity, banana, sweet pear), pentyl acetate  
1101 451 (fruity, banana, pear and apples notes), (Z)-2-hexenyl acetate (fruity odour), 2-  
1102 452 methylpropyl butanoate (fruity, sweet, pineapple, apple, and tutti-frutti notes), butyl  
1103 453 hexanoate (fruity, pineapple, and ripe fruit notes), 3-methyl-2-butanol (alcoholic, spicy,  
1104 454 ethereal, cognac, fruity, fresh odour), 1-butanol (fruity, sweet, banana, fruit juice, and  
1105 455 tutti-frutti notes), 2-methyl-1-butanol (wine, onion, fruity, alcoholic, and whisky notes), 1-  
1106 456 hexanol (herbal, fatty, and fruity), (E)-2-hexen-1-ol (green leafy, fresh, fatty, grassy with  
1107 457 fruity and juicy nuances), hexanal (green, woody, vegetative, apple, grassy, citrus and  
1108 458 orange with a fresh, lingering aftertaste) and acetone (fruity, blueberry, raspberry, and

1122  
1123  
1124 459 berry notes). Among the mentioned compounds, there are some that are common in the  
1125  
1126 460 volatile profiles of pears (methyl acetate, 3-methylbutyl acetate, 1-butanol, 1-hexanol,  
1127  
1128 461 and hexanal); therefore, increases in their contents could enhance flavour consumer's  
1129  
1130 462 perception. Nevertheless, we were not able to carry out a consumer preference test as  
1131  
1132 463 this strain is not yet included in the QPS (Qualified Presumption of Safety) list of the  
1133  
1134 464 EFSA.

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1137 465

## 1138 1139 466 **5. Conclusions**

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1142 467 To conclude, CPA-7 was able to control the growth of *L. monocytogenes* after 9 days of  
1143  
1144 468 storage. On the other hand, no effect was observed on the *S. enterica* population under  
1145  
1146 469 the tested conditions. These results suggested that CPA-7 did not have a bactericidal  
1147  
1148 470 effect against foodborne pathogens. CPA-7 treatment could improve the volatile profile  
1149  
1150 471 and did not negatively affect the fruit quality. We did not observe a clear effect of  
1151  
1152 472 postharvest CaCl<sub>2</sub> treatment on the efficacy of CPA-7, and we studied the quality  
1153  
1154 473 parameters of fresh-cut pear.

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**Table 1.** Headspace gas composition (O<sub>2</sub> and CO<sub>2</sub>, KPa) inside fresh-cut pear trays stored at 5 °C ± 1 °C treated (Ca) or not (None) with 1 % CaCl<sub>2</sub> after harvest and inoculated (CPA-7) or not (Control) with 10<sup>8</sup> cfu mL<sup>-1</sup> of *P. graminis* CPA-7 after cutting. Different capital letters in the same row indicate significant differences during storage time according to a Tukey test (P<0.05) and different lowercase letters in the same column indicate significant differences between different treatment at the same time sampling for each gas according to a Tukey test (P<0.05).

	Postharvest treatment	Biopreservation treatment	Days			
			0	2	6	9
O <sub>2</sub>	None	None (Control)	21.0 ± 0.0 Ax	14.7 ± 0.5Bx	15.6 ± 1.4 Bx	14.6 ± 0.1 Bx
		CPA-7 (CPA-7)	21.0 ± 0.0 Ax	15.8 ± 0.6 Bx	14.7 ± 0.2 Bx	14.3 ± 1.0 Bx
	CaCl <sub>2</sub>	None (CaControl)	21.0 ± 0.0 Ax	14.8 ± 1.9 Bx	16.4 ± 1.4 Cx	12.6 ± 1.0 Cx
		CPA-7 (CaCPA7)	21.0 ± 0.0 Ax	15.7 ± 0.4 Bx	15.0 ± 1.6 Bx	13.0 ± 2.0 Bx
CO <sub>2</sub>	None	None (Control)	0.0 ± 0.0 Bx	6.1 ± 0.2 Ax	6.6 ± 1.4 Ax	7.9 ± 0.1 Ax
		CPA-7 (CPA-7)	0.0 ± 0.0 Cx	4.9 ± 0.4 By	7.1 ± 0.3 Ax	7.8 ± 0.9 Ax
	CaCl <sub>2</sub>	None (CaControl)	0.0 ± 0.0 Cx	5.7 ± 0.7 Bxy	5.9 ± 1.5 Bx	9.7 ± 0.8 Ax
		CPA-7 (CaCPA7)	0.0 ± 0.0 Cx	4.7 ± 0.1 By	7.0 ± 1.4 ABx	9.3 ± 1.8 Ax

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 1537 **Table 2.** Solids soluble content (SSC, %) and titratable acidity (TA, g L<sup>-1</sup>) produced on  
 1538 fresh-cut pear stored at 5 °C treated (Ca) or not (None) with 1 % CaCl<sub>2</sub> after harvest  
 1539 629 and inoculated (CPA-7) or not (Control) with 10<sup>8</sup> cfu mL<sup>-1</sup> of *P. graminis* CPA-7 after  
 1540 630 cutting. Different capital letters in the same row indicate significant differences within  
 1541 631 the same treatment along the storage time according to Tukey's test (P < 0.05).  
 1542 632 Different lower case letters in the same column indicate significant differences between  
 1543 633 treatments at each sampling time according to Tukey's test (P < 0.05).  
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	Postharvest treatment	Biopreservation treatment	Days at 5°C			
			0	2	6	9
SSC (%)	None	None (Control)	13.9 ± 0.0 Cb	14.0 ± 0.1 ABb	13.9 ± 0.1 BCb	14.1 ± 0.1 Ac
		CPA-7 (CPA-7)	13.0 ± 0.0 Cc	13.7 ± 0.1 Ac	13.4 ± 0.1 Bc	13.1 ± 0.1 Cd
	CaCl <sub>2</sub>	None (CaControl)	14.5 ± 0.1 Ba	14.5 ± 0.1 Ba	14.2 ± 0.1 Ca	14.8 ± 0.0 Aa
		CPA (CaCPA-7)	13.9 ± 0.0 Bb	14.2 ± 0.1 Ab	14.3 ± 0.0 Aa	14.3 ± 0.1 Ab
TA (g L <sup>-1</sup> )	None	None (Control)	1.99 ± 0.07 Aa	2.10 ± 0.11 Aa	1.70 ± 0.08 Bab	1.70 ± 0.07 Ba
		CPA-7 (CPA-7)	1.95 ± 0.08 Aa	2.14 ± 0.18 Aa	1.89 ± 0.08 Aa	1.27 ± 0.10 Bb
	CaCl <sub>2</sub>	None (CaControl)	1.99 ± 0.05 Aa	1.94 ± 0.12 Aa	1.63 ± 0.08 Bb	1.60 ± 0.03 Ba
		CPA-7 (CaCPA-7)	1.83 ± 0.13 Aba	1.94 ± 0.14 Aa	1.62 ± 0.05 Bb	1.58 ± 0.04 Ba

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639 **Table 3.** Volatile compounds (ng g<sup>-1</sup>) produced by minimally processed pear stored at 5  
640 °C treated with CaCl<sub>2</sub> after harvest. Different capital letters indicate significant differences  
641 between pear wedges treated and untreated with CPA-7 the same sampling time  
642 according to Student's t test at significance level of P < 0.05. nd: not detected. traces:  
643 ≤ 10 ng g<sup>-1</sup>

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Volatile compounds	Treated with CaCl <sub>2</sub>					
	0 days		2 days		6 days	
	CPA-7	no CPA-7	CPA-7	no CPA-7	CPA-7	no CPA-7
<b>ACETATES</b>						
Methyl acetate	899,9 A	106,7 B	107,6 A	nd	1379,2 A	nd
Ethyl acetate	2813,8 A	1388,5 B	2488,7 A	2122,8 B	1172,7 A	707,5 B
Propyl acetate	127,7 B	296,8 A	251,8 B	449,0 A	492,4 B	3538,6 A
Butyl acetate	4746,0 B	13545,4 A	10425,1 B	22699,3 A	9860,3 A	4635,0 B
3-Methylbutyl acetate	nd	417,3 A	144,3 B	211,8 A	168,7 B	1589,4 A
Pentyl acetate	226,0 B	356,8 A	156,8 A	traces B	538,4 B	10044,7 A
Hexyl acetate	6266,3 B	6414,3 A	4872,9 B	7838,3 A	8269,8 B	13127,6 A
(Z)-2-hexenyl acetate	271,8 A	traces B	188,8 A	traces B	87,4 B	1943,1 A
Octyl acetate	532,3 A	46,3 B	619,0 A	64,7 B	164,6 B	1166,8 A
<b>BUTANOATES</b>						
Methyl butanoate	433,1 A	89,2 B	372,8 A	nd	131,9 B	2742,5 A
Ethyl 2-methylbutanoate	58,4 B	83,6 A	85,0 A	nd	8789,8 A	1931,7 B
2-Methylpropyl butanoate	246,5 B	525,8 A	664,9 A	662,1 A	596,0 B	2105,9 A
Butyl 2-methylbutanoate	nd	nd	nd	nd	158,5 B	1558,2 A
Butyl butanoate	70,1 A	71,6 A	154,1 B	242,0 A	207,1 A	134,5 B
2-Methylbutyl-2-methylbutanoate	149,6 B	357,1 A	nd	416,8 A	nd	5884,4 A
Hexyl butanoate	nd	nd	nd	nd	nd	nd
Hexyl 2-methylbutanoate	nd	nd	nd	nd	nd	nd
<b>HEXANOATES</b>						
Ethyl hexanoate	nd	nd	nd	nd	782,1 B	2791,0 A
Butyl hexanoate	475,2 A	nd	1235,3 B	1471,1 A	604,4 B	7734,6 A
Pentyl hexanoate	116,6 A	nd	190,4 A	nd	nd	1097,6 A
Hexyl hexanoate	392,9 A	334,7 A	280,3 A	nd	188,8 B	2696,4 A
<b>PROPANOATES</b>						
<i>tert</i> -Butyl propanoate	137,5 A	97,7 B	94,5 A	nd	256,7 B	2198,5 A
Butyl propanoate	148,2 A	45,9 B	238,6 A	nd	89,9 B	1759,6 A
<b>OCTANOATES</b>						
Ethyl octanoate	956,6 A	nd	287,4 A	67,0 B	nd	815,1 A
<b>PENTANOATES</b>						
Pentyl 3-methylbutanoate	nd	179,2 A	nd	236,8 A	nd	1800,1 A
<b>ALCOHOLS</b>						
Ethanol	24582,3 B	61475,7 A	6170,3 B	8099,1 A	2971,8 B	11412,8 A
3-Methyl-2-butanol	11972,9 B	14742,8 A	11672,9 A	nd	12480,4 A	4561,2 B
1-Butanol	59,1 B	81,6 A	75,4 A	nd	105,4 B	2729,8 A
2-Methyl-1-butanol	nd	73,1 A	nd	nd	205,0 B	731,9 A
1-Pentanol	166,5 B	278,0 A	nd	109,3 A	60,6 B	1588,3 A
1-Hexanol	233,0 A	nd	304,5 A	nd	261,3 B	1122,5 A
(E)-2-Hexen-1-ol	traces B	396,1 A	traces A	traces A	traces A	traces A
2-Ethyl-1-hexanol	3674,8 A	1197,0 B	3439,4 A	723,7 B	793,4 B	9456,4 A
1-Octanol	94,8 A	59,0 B	181,1 A	75,4 B	nd	170,7 A
Benzyl alcohol	2809,1 A	329,7 B	6167,3 A	664,0 B	304,3 B	10877,9 A
<b>ALDEHYDES</b>						
Acetaldehyde	1139,8 A	985,5 B	673,4 A	nd	nd	693,4 A
Hexanal	714,0 A	594,1 B	37316,8 A	2546,8 B	1713,9 B	4962,6 A
2-Ethylhexanal	52,4 B	260,2 A	78,0 B	1127,0 A	69,3 B	1048,3 A
Benzaldehyde	352,7 A	nd	489,1 A	202,8 B	201,2 B	1402,2 A
<b>TERPENES</b>						
$\alpha$ -Farnesene	577,4 A	50,6 B	583,3 A	135,9 B	776,9 B	1188,2 A
<b>KETONES</b>						
Acetone	531,4 A	traces B	traces A	traces A	traces A	traces A
6-Methyl-5-hepten-2-one	nd	nd	nd	nd	nd	nd
<b>ACIDS</b>						
Acetic acid	252,5 A	nd	583,8 A	90,1 B	nd	2905,3 A

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1773 **Table 4.** Volatile compounds produced (ng g<sup>-1</sup>) by minimally processed pear stored at 5  
1774 °C untreated with CaCl<sub>2</sub> after harvest. Different capital letters indicate significant  
1775 differences between pear wedges treated and untreated with CPA-7 the same sampling  
1776 648 time according to Student's t test at significance level of P < 0.05. nd: not detected.  
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1778 650 traces: ≤ 10 ng·g<sup>-1</sup>

Volatile compounds	Untreated with CaCl <sub>2</sub>					
	0 days		2 days		6 days	
	CPA-7	no CPA-7	CPA-7	no CPA-7	CPA-7	no CPA-7
<b>ACETATES</b>						
Methyl acetate	258,0 A	107,0 B	traces A	traces A	traces A	traces A
Ethyl acetate	609,3 B	1347,3 A	1520,5 B	1649,0 A	782,0 B	2628,8 A
Propyl acetate	315,9 A	395,7 A	556,5 A	503,1 A	484,8 A	289,0 B
Butyl acetate	9768,8 A	5127,2 B	18653,7 A	18972,4 A	13518,8 B	14022,1 A
3-Methylbutyl acetate	237,7 A	nd	274,2 A	nd	187,5 A	nd
Pentyl acetate	nd	17,8 A	nd	nd	nd	nd
Hexyl acetate	8301,7 A	5043,2 B	11384,6 A	6844,1 B	5776,1 B	7945,7 A
(Z)-2-hexenyl acetate	351,5 A	traces B	292,8 A	traces B	245,9 A	traces B
Octyl acetate	1348,6 A	332,7 B	173,1 A	nd	259,5 A	nd
<b>BUTANOATES</b>						
Methyl butanoate	187,3 A	nd	68,6 B	224,4 A	132,1 A	nd
Ethyl 2-methylbutanoate	nd	nd	367,8 A	nd	nd	185,1 A
2-Methylpropyl butanoate	704,4 A	115,4 B	824,5 A	753,9 B	933,9 A	487,3 B
Butyl 2-methylbutanoate	nd	nd	nd	nd	nd	nd
Butyl butanoate	230,9 A	traces B	385,1 A	traces B	558,9 A	407,2 B
2-Methylbutyl-2-methylbutanoate	nd	nd	nd	303,1 A	nd	nd
Hexyl butanoate	373,6 A	nd	nd	nd	nd	nd
Hexyl 2-methylbutanoate	577,3 A	nd	nd	nd	nd	nd
<b>HEXANOATES</b>						
Ethyl hexanoate	nd	nd	123,4 A	nd	nd	nd
Butyl hexanoate	334,8 A	nd	1125,9 A	nd	920,9 A	nd
Pentyl hexanoate	478,3 A	nd	nd	nd	nd	nd
Hexyl hexanoate	1006,4 A	599,7 B	466,8 A	254,0 B	296,5 A	nd
<b>PROPANOATES</b>						
<i>tert</i> -Butyl propanoate	nd	115,7 A	nd	nd	nd	nd
Butyl propanoate	222,1 A	nd	116,8 A	nd	331,4 A	nd
<b>OCTANOATES</b>						
Ethyl octanoate	437,1 A	nd	nd	119,6 A	51,0 A	nd
<b>PENTANOATES</b>						
Pentyl 3-methylbutanoate	nd	nd	87,9 A	nd	nd	406,4 A
<b>ALCOHOLS</b>						
Ethanol	188734,4 A	3676,9 B	13305,8 A	6648,5 B	1983,8 B	6586,0 A
3-Methyl-2-butanol	221,3 B	15256,4 A	391,2 A	nd	nd	321,6 A
1-Butanol	nd	118,9 A	nd	nd	nd	nd
2-Methyl-1-butanol	nd	nd	114,2 A	nd	nd	nd
1-Pentanol	nd	nd	nd	nd	nd	122,2 B
1-Hexanol	291,0 A	nd	406,8 A	nd	368,7 A	nd
(E)-2-Hexen-1-ol	traces A	traces A	traces A	traces A	767,0 A	traces B
2-Ethyl-1-hexanol	5575,7 A	2445,7 B	1141,2 A	516,4 B	1133,4 A	304,1 B
1-Octanol	506,4 A	104,4 B	182,4 A	nd	106,0 A	nd
Benzyl alcohol	7283,4 A	172,5 B	2685,9 A	1239,0 B	4129,8 A	442,4 B
<b>ALDEHYDES</b>						
Acetaldehyde	2122,5 A	780,4 B	401,4 B	834,5 A	nd	1262,8 A
Hexanal	1521,9 A	nd	2813,0 A	1381,0 B	6668,4 A	1515,8 B
2-Ethylhexanal	56,3 A	59,3 A	156,1 A	88,9 B	110,9 A	78,4 B
Benzaldehyde	1123,0 A	649,4 B	399,8 A	nd	405,5 A	nd
<b>TERPENES</b>						
α-Farnesene	3658,5 A	931,7 B	264,6 A	traces B	531,9 A	traces B
<b>KETONES</b>						
Acetone	traces B	62,9 A	traces A	traces A	traces A	traces A
6-Methyl-5-hepten-2-one	405,3 A	nd	119,3 A	nd	nd	nd
<b>ACIDS</b>						
Acetic acid	775,9 A	nd	nd	nd	164,9 A	nd

1830  
1831  
1832 652  
1833  
1834 653 **FIGURE CAPTIONS**  
1835

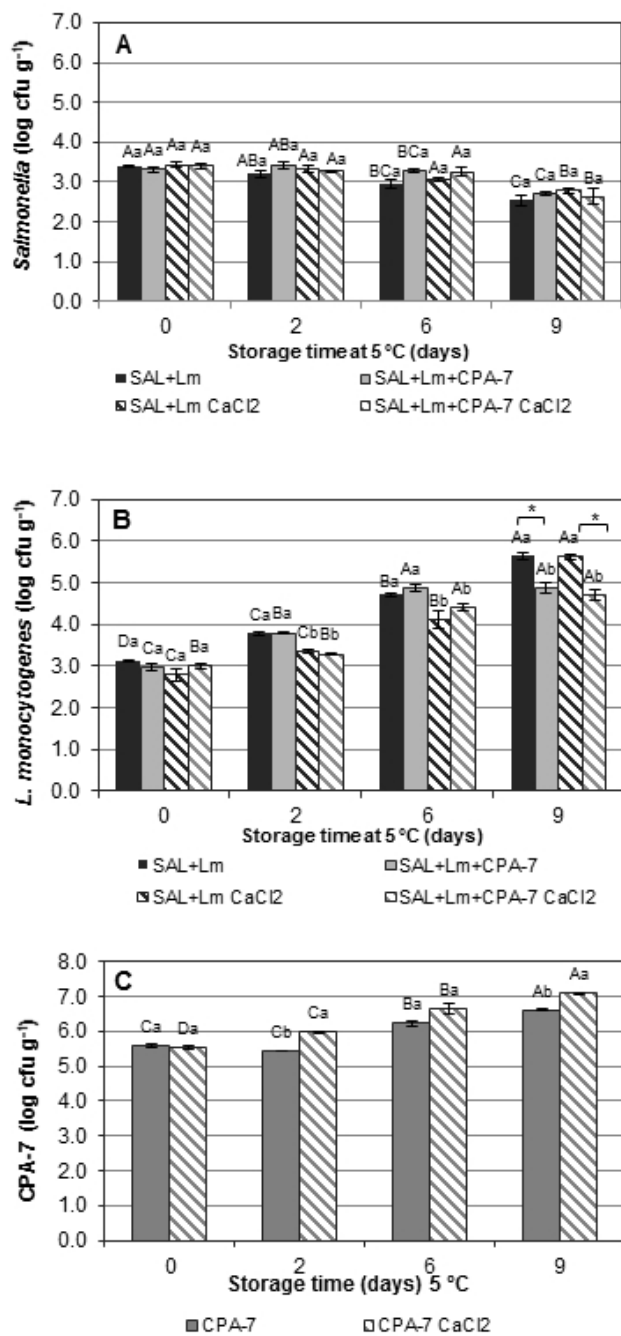
1836 654 **Fig. 1** *Salmonella* (A), *L. monocytogenes* (B) and CPA-7 (C) population (log cfu g<sup>-1</sup>) on  
1837  
1838 655 fresh-cut pear treated or not with CaCl<sub>2</sub> 1 % after harvest and then processed and stored  
1839  
1840 656 at 5 ± 1 °C. The results are the means of three values. Vertical bars indicate the standard  
1841  
1842 657 deviations of the means. Different capital letters indicate significant differences within the  
1843  
1844 658 same treatment throughout the storage time according to Tukey's test (P < 0.05).  
1845  
1846 659 Different lower-case letters indicate significant differences among the same treatment on  
1847  
1848 660 pears untreated or treated with CaCl<sub>2</sub> at each sampling time according to Student's t test  
1849  
1850 661 (P < 0.05). \* Indicates significant differences between samples with or without CPA-7 at  
1851  
1852 662 each sampling time (Student's t test at significance level of P < 0.05).  
1853

1854 663 **Fig. 2** Concentration (mL·L<sup>-1</sup>) of ethanol (A) and acetaldehyde (B) produced on CaCl<sub>2</sub>-  
1855  
1856 664 untreated pear wedges inoculated without CPA-7 or with CPA-7 and CaCl<sub>2</sub>-treated pear  
1857  
1858 665 wedges inoculated without CPA-7 or with CPA-7 processed and stored at 5 ± 1 °C. The  
1859  
1860 666 results are the means of 3 values. Vertical bars indicate the standard deviations of the  
1861  
1862 667 means. Different capital letters indicate significant differences within the same treatment  
1863  
1864 668 along the storage time according to Tukey's test (P < 0.05). Different lower-case letters  
1865  
1866 669 indicate significant differences between treatments at each sampling time according to  
1867  
1868 670 Tukey's test (P < 0.05).  
1869  
1870

1871 671 **Fig. 3** Score (A) and loading (B) plots of PC1 vs. PC2 corresponding to a PLSR model  
1872  
1873 672 for CPA-7 population vs. emissions of volatile compounds on pear wedges stored at 5°  
1874  
1875 673 C.  
1876

1877 674 **Fig. 4** Regression coefficients corresponding to a PLSR model for CPA-7 population vs.  
1878  
1879 675 emissions of volatile compounds on pear wedges stored at 5 ± 1 °C.  
1880  
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1882 676  
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Figure 1



**Figure 2**

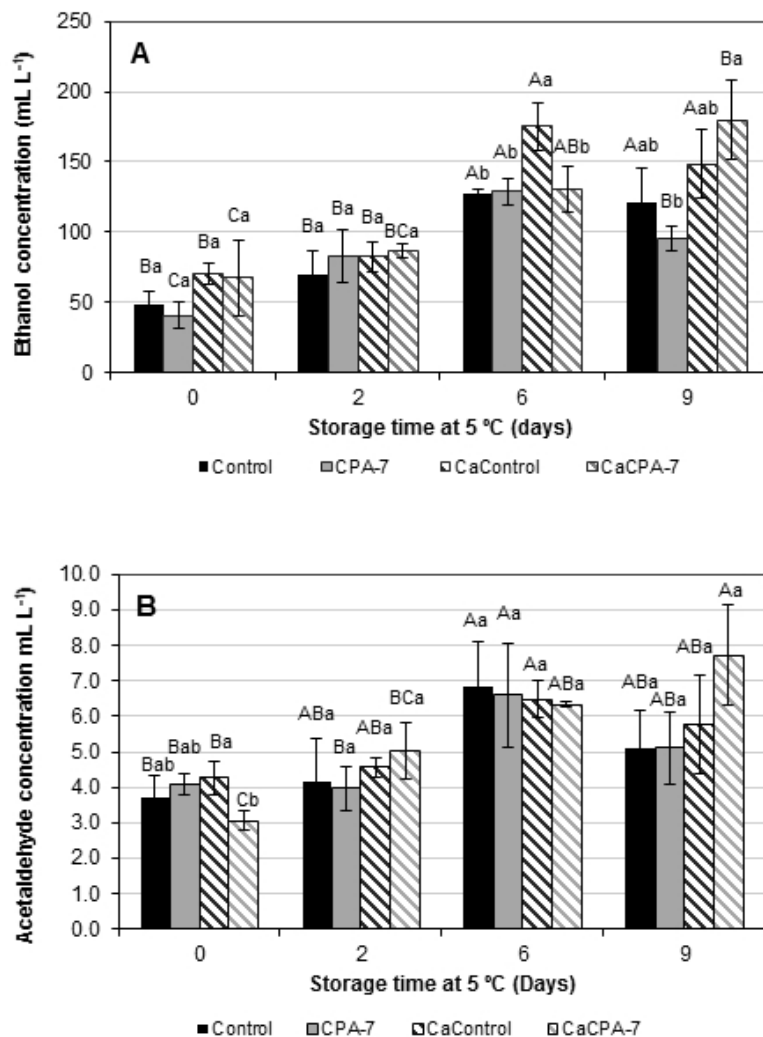
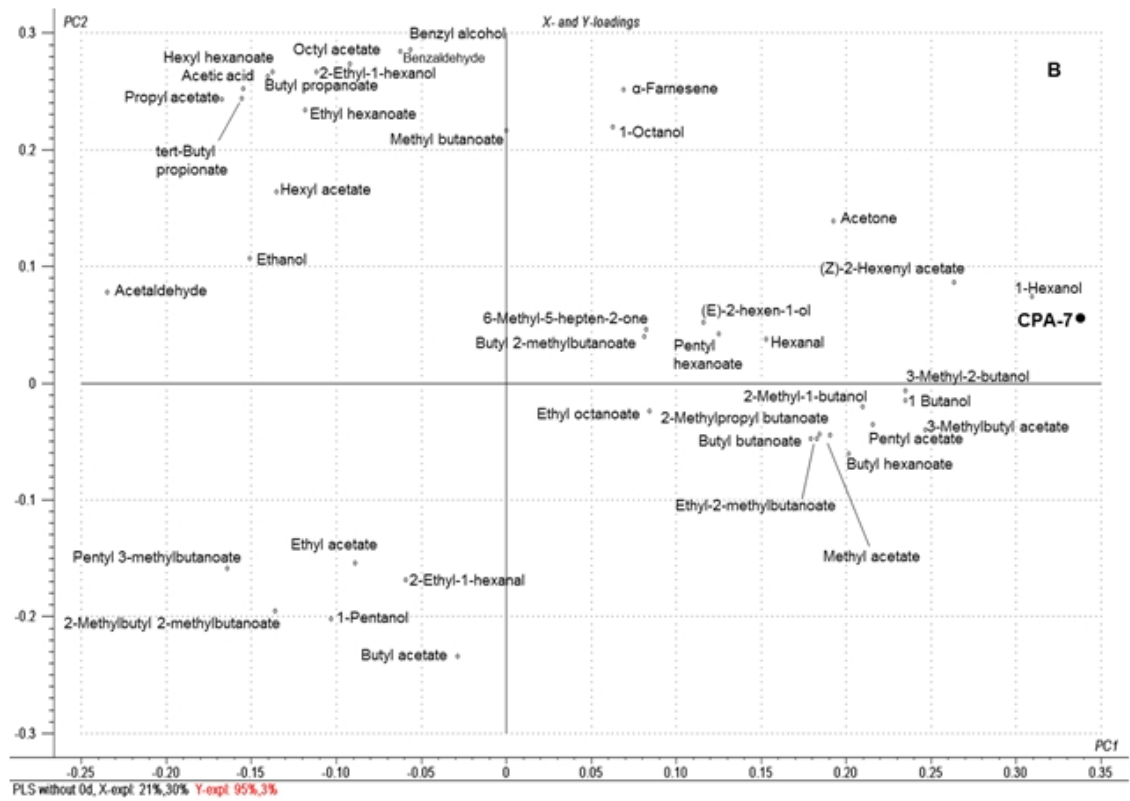
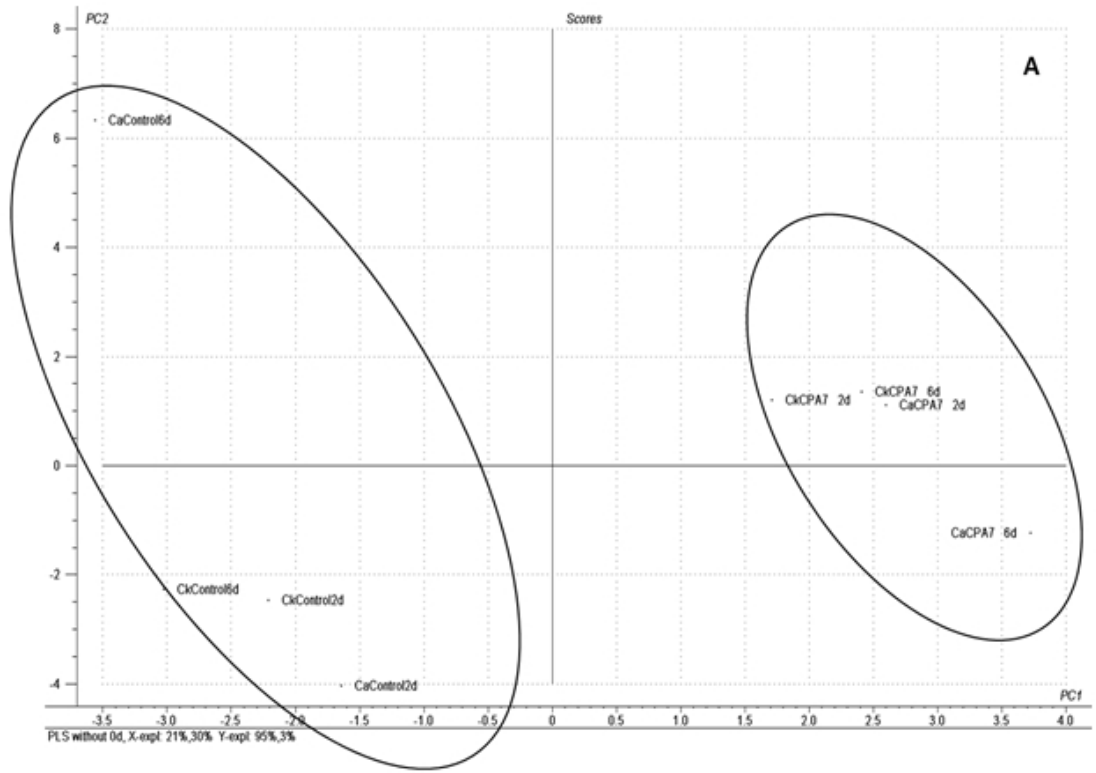


Figure 3



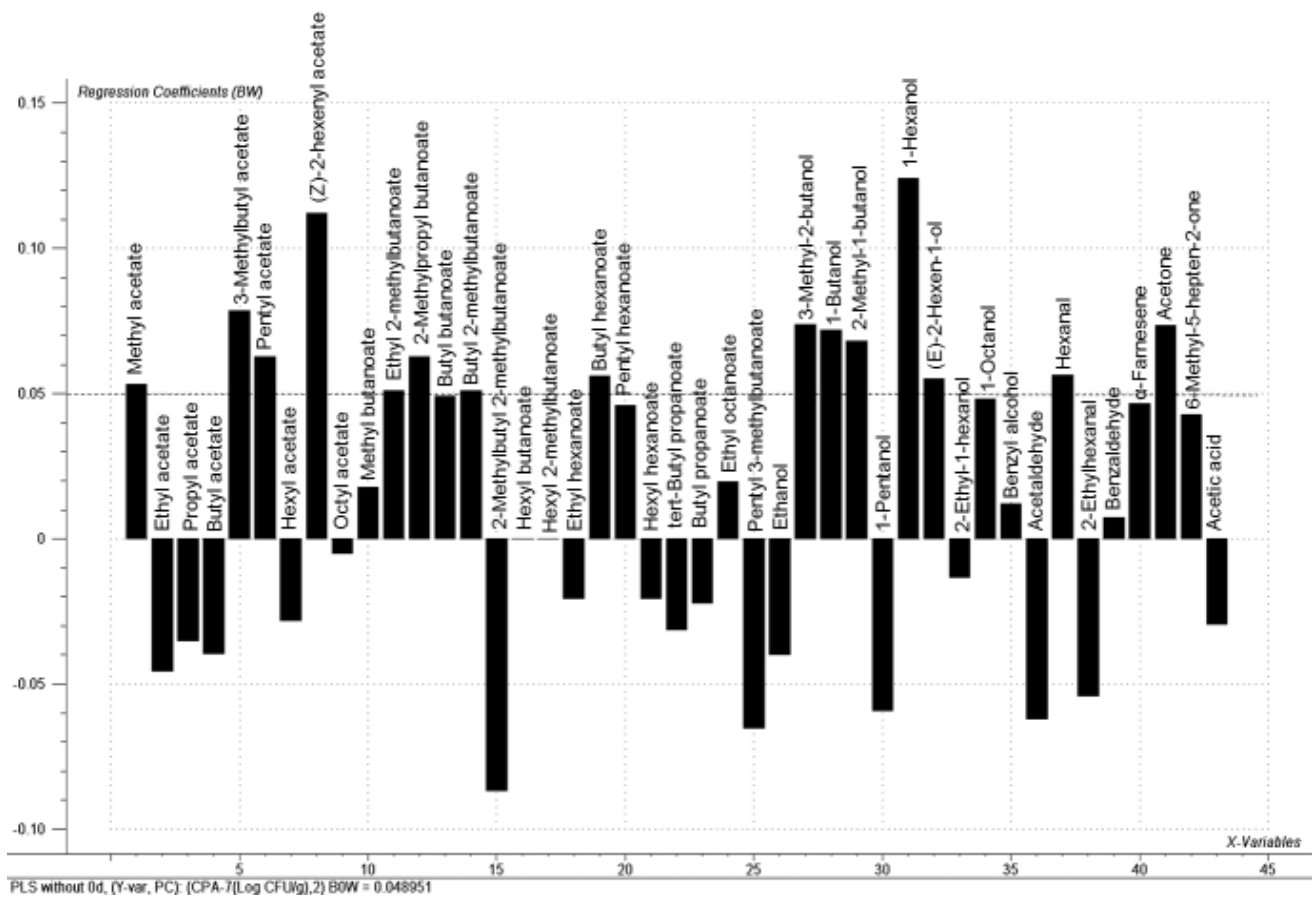


Figure 4