1   **Biopreservation of fresh-cut pear using *Lactobacillus rhamnosus* GG and effect**

2   **on quality and volatile compounds**

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13

14   Abstract

15   In recent years, the consumption of minimally processed fruit has increased. However,

16   unfortunately, these products could be an appropriate vehicle for the transmission of

17   foodborne pathogens. In this study, the antagonistic capacity of the probiotic strain

18   *Lactobacillus rhamnosus* GG against a cocktail of 5 serovars of *Salmonella* and 5

19   serovars of *Listeria monocytogenes* on fresh-cut pear at conditions simulating

20   commercial application was assessed. Moreover, its effect on fruit quality, particularly

21   on the volatile profile, was determined, during 9 days of storage at 5 °C. *L.*

22   *mo*nocytogenes population was reduced by approximately 1.8 log-units when co-

23   inoculated with *L. rhamnosus* GG. However, no effect was observed in *Salmonella*.

24   Fruit quality (soluble solids content and titratable acidity) did not change when the

25   probiotic was present. A total of 48 volatile compounds were identified using gas

26   chromatography. Twelve of the compounds allowed to discriminate *L. rhamnosus* GG-

27   treated and untreated pears. Considering their odour descriptors, their increases could

28   be positive in the flavour perception of *L. rhamnosus* GG-treated pear. The probiotic

29   was able to control *L. monocytogenes* population on fresh-cut pear, which could be a

30   vehicle of probiotic microorganisms as quality of fruit was not affected when the

31   probiotic was present.

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33   Keywords: *Salmonella*; *Listeria*; probiotic; biocontrol; food safety

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40   1. Introduction

41   Ready-to-eat fruits and vegetables are increasingly popular products, mainly due to the

42 fact that they are easy to consume, and also fresh and healthy because of their

43 nutritional contribution (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). Fresh fruits

44 are generally considered to be microbiologically safe. However, they could be

45 contaminated in the preharvest environment due to the irrigation water, air, compost,

46 animals, human handling … and also during harvest and postharvest (Beuchat, 1995).

47 Moreover, when fruit is processed, bacteria may be transferred from external fruit

48 surfaces to edible portions, being a potential vehicle for the transmission of the main

49 foodborne pathogens such as *Salmonella*, *Escherichia coli* or *Listeria monocytogenes*

50 (Ukuku, Geveke, Chau, & Niemira, 2016). *L. monocytogenes* is able to grow at

51 refrigerated temperature on fresh cut apple (Alegre, Viñas, Usall, Anguera, & Abadias,

52 2011), melon (Abadias et al., 2014) and on melon, apple and mango at 7 °C (Lokerse,

53 Maslowska-Corker, van de Wardt, & Wijtzes, 2016). Moreover, controlled atmosphere

54 storage does not appear to influence growth rates (Oliveira, Abadias, Colas-Meda,

55 Usall, & Viñas, 2015).

56 In order to reduce pathogenic microorganisms, different techniques have been studied,

57 one of which is biopreservation using lactic acid bacteria (LAB). LAB are able to

58 convert lactose and other sugars in lactic acid and could generate other final

59 metabolites such as ethanol if they carry out a heterolactic fermentation (Li, 2004).

60 Another characteristic of this genus is that most of the bacteria which are included in it

61 are considered to be probiotics. According to reports by FAO/WHO (2002), probiotics

62 are defined as living microorganisms that, when administered in adequate amounts,

63 confer benefits to host health, through a positive action of intestinal microbiota. The

64 way in which probiotics provide beneficial effects on health is, mainly, by activating the

65 immune system, improving intestinal microbial balance and controlling foodborne

66 pathogens. Some LAB also have antimicrobial activity, which is carried out by secreting

67 antimicrobial byproducts, such as lactic acid, hydrogen peroxide and polypeptides,

68 inhibiting or blocking adhesion to epithelial cells and the invasion abilities of

69 enteropathogens (Ng, Hart, Kamm, Stagg, & Knight, 2009; Peng, Reichmann, &

70   Biswas, 2015). Some probiotic bacteria have demonstrated a good ability to reduce the

71   level of foodborne pathogens on fresh-cut fruit. Russo et al. (2014; 2015) demonstrated

72   that some probiotic strains have an antagonistic effect against *L. monocytogenes* on

73   fresh-cut pineapple and melon and Siroli et al. (2015) demonstrated the same effect on

74   fresh-cut apples. *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG) demonstrated to

75   have a bacteriostatic effect against *L. monocytogenes* and *Salmonella* on fresh-cut

76   apple (Alegre et al., 2011) and pear (Iglesias, Abadias, Anguera, Sabata, & Viñas,

77   2017). However, little is known about the effect of the application of this probiotic strain

78   on the quality of fresh-cut fruit and, in particular, on the volatile compounds (VCs)

79   (Rößle, Brunton, Gormley, Ross, & Butler, 2010b).

80   Salmeron, Loeza-Serrano, Perez-Vega, & Pandiella (2015) studied VCs produced by

81   three different lactobacilli in barley and malt fermentation and they observed that the

82   VC profile varies, depending on the matrix. The VC profile can also provide desirable

83   sensorial notes for the consumer, contributing to the characteristic flavour and aroma in

84   determinate foods (Sreekumar, Al-Attabi, Deeth, & Turner, 2009). In the case of

85   lactobacilli fermentations, VCs such as ethanol, acetaldehyde, acetone, diacetyl, and

86   ethyl acetate are produced and which are responsible for the flavour in specific foods

87   and beverages (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003; Salmeron et

88   al., 2015). Nevertheless, the same VCs could cause off-flavour notes and non-pleasant

89   flavours in some matrix food (Kopsahelis, Kanellaki, & Bekatorou, 2007). It is important

90   to know about the evolution of quality attributes of fresh-cut products, such as odour,

91   taste, colour and texture in order to relate with microbiological and physiological

92   features during the product storage.

93   The combination of probiotic strains with fruit could be promising due to the fact that it

94   could be one way to help vegetarians, vegans and people who are allergic to dairy food

95   to ingest these bacteria from alternative sources and obtain their benefits (Luckow &

96   Delahunty, 2004).

97   The aim of this study was to evaluate the effect of the application of *L. rhamnosus* GG

98   on the quality of fresh-cut pear at conditions simulating commercial application with

99   special emphasis on the volatile compounds. Pears were treated or not-treated with

100   $CaCl_2$ after harvest and stored in controlled atmosphere (CA) conditions before

101   processing. The antagonistic effect of *L. rhamnosus* GG against *L. monocytogenes* and

102   *Salmonella* was validated. To the best of our knowledge, this study is the first to

103   evaluate sensorial aspects of fresh-cut pear treated with a probiotic strain simulating

104   commercial conditions.

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106

107   2. Material and methods

108   2.1. Fruit

109   'Conference' pears (*Pyrus communis* L. cv. Conference) were used in this experiment.

110   After harvest, pears were divided into two lots. Whole fruits of lot 1 were dipped in

111   water at 25 °C for 5 min and this group was used as control. Whole fruits of lot 2 were

112   dipped in a solution containing 10 g L^{-1} $CaCl_2$ at 25 °C during 5 min. After fruit harvest,

113   cold storage and CA are essential to delay the ripening process. In apples, postharvest

114   dipping in $CaCl_2$ before storage contribute to extending commercial life in whole fruit as

115   well as minimally processed (MP) fruit.

116   Afterwards, pears of both lots were stored at 0 ± 1 °C during 8 months in CA (2 kPa O_2

117   and 1 kPa CO_2) up to the time of the experiment. After this storage time, the pears

118   were conditioned at 20 °C until the optimum ripeness stage for processing (44 ± 3.2 N)

119   (Soliva-Fortuny, Alos-Saiz, Espachs-Barroso, & Martin-Belloso, 2004).

120

121   2.2. Bacterial strains and inoculum preparation

122   A cocktail of five serovars of *Salmonella enterica* subsp. *enterica* was used: Agona

123   (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC BAA-710),

124   Gaminara (ATCC BAA-711) and Enteritidis (CECT 4300). Each *Salmonella* strain was

125   grown individually in tryptone soy broth (TSB, Oxoid) medium for 20-24 h at 37 ± 1 °C.

126   A cocktail of five *Listeria monocytogenes* serovars was used: serovar 1a (CECT 4031),

127   serovar 3a (CECT 933); serovar 4d (CECT 940), serovar 4b (CECT 4032) and serovar

128   1/2a, which was previously isolated in our laboratory from a fresh-cut lettuce sample

129   (Abadias, Usall, Anguera, Solsona, & Viñas, 2008). *L. monocytogenes* strains were

130   grown individually in TSB supplemented with 6 g L^{-1} of yeast extract (tryptone yeast

131   extract soy broth, TSBYE) for 20-24 h at 37 ± 1 °C. Bacterial cells were harvested by

132   centrifugation at 9800 x g, 10 min at 10 °C. The broth was decanted and the cells were

133   resuspended in saline solution (SS; 8.5 g L^{-1} NaCl). Equal volumes of the five

134   *Salmonella* concentrated suspensions were mixed to produce a single suspension, as

135   well as the five *L. monocytogenes* concentrated suspensions.

136   The antagonist used in this study was the probiotic strain *Lactobacillus rhamnosus* GG

137   (ATCC 53103) (*L. rhamnosus* GG) (from Ashtown Food research Centre, Teagasc,

138   Ashtown, Dublin, Ireland). The antagonist was grown in de Man, Rogosa and Sharpe

139   (MRS, Biokar Diagnostics, Beauvais, France) broth for 20-24 h at 37 ± 1 °C. Bacterial

140   cells were harvested by centrifugation at 9800 x g, 10 min at 10 °C. The broth was

141   decanted and the cells were resuspended in sterile distilled water.

142   For the inoculum preparation, an aliquot of each of the bacterial concentrated

143   suspensions was added to deionised water to obtain approximately 10^5 CFU mL^{-1} in

144   the case of *Salmonella* and *L. monocytogenes* and 10^8 CFU mL^{-1} for *L. rhamnosus*

145   GG. Inoculum concentration was checked by plating appropriate dilutions onto XLD

146   (Xylose-Lysine-Desoxycholate Agar, Oxoid) for *Salmonella*, onto Palcam agar (Palcam

147   Agar Base with selective supplement, Biokar Diagnostics, Beauvais, France) for

148   *L. monocytogenes* and onto MRS agar for *L. rhamnosus* GG. The plates were

149   incubated at 37 ± 1 °C for 24 and 48 h for *Salmonella* and *L. monocytogenes*,

150   respectively, and at 37 ± 1 °C for 48 h for the probiotic strain.

151

152   2.3. Inoculation of fruit and packaging

153   Prior to the experimental study, pears of both lots were washed in running tap water

154   and surface disinfected with ethanol at 70 %. They were peeled and cut into 10 wedges

155   using a handheld apple slicer/corer. An antioxidant solution containing 20 g L^{-1} ascorbic

156   acid, 20 g L^{-1} sodium citrate and 10 g L^{-1} $CaCl_2$ was used to prevent fresh-cut pear

157   browning. Previous studies (data not shown) demonstrated that this antioxidant

158   solution has no effect on bacteria viability. Pear wedges were dipped (1:2 w/v) for 2 min

159   at 150 rpm according to the following treatments: (a) control (untreated): antioxidant

160   solution (b) Sal + Lm: antioxidant solution inoculated with *Salmonella* and *L.*

161   *monocytogenes* at 10^5 CFU mL^{-1} each, (c) *L. rhamnosus* GG: antioxidant solution

162   inoculated with *L. rhamnosus* GG at 10^8 CFU mL^{-1} each or (d) Sal + Lm + *L.*

163   *rhamnosus* GG: antioxidant solution inoculated with *Salmonella* and *L. monocytogenes*

164   (10^5 CFU mL^{-1}) and *L. rhamnosus* GG (10^8 CFU mL^{-1}). Afterwards, they were allowed

165   to dry in a laminar flow biosafety cabinet.

166   Pear wedges were packaged (110 ± 5 g) in passive atmosphere by placing them in

167   375-mL polypropylene trays and sealing with a non-peelable polypropylene plastic film

168   (PP-110, ILPRA, Italy) of 64 μm in thickness with an O_2 permeability of 110 cm^3 m^{-2}

169   day^{-1} atm^{-1} at 23 °C and a water steam permeability of 10 g m^{-2} day^{-1} at 23 °C and 90 %

170   relative humidity (ILPRA, Italy). Pear trays were stored at 5 °C. The samples were

171   examined on the day of inoculation and after 2, 6 and 9 days.

172

173   2.4. Enumeration of microorganisms in pear wedges

174   Populations of *Salmonella*, *L. monocytogenes* (in treatments b and d) and *L.*

175   *rhamnosus* GG (in treatments c and d) were determined in three sample trays at each

176   sampling time. For the analysis, 10 g of pear from each tray were mixed with 90 mL of

177   buffered peptone water (BPW, Oxoid LTD, Basingstoke, Hampshire, England) in a

178   sterile bag and homogenised in a Stomacher® 400 (Seward, London, UK) set at 230

179   strokes min^{-1} for 2 min. A further set of ten-fold dilutions was made with saline peptone

180 (SP; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone) and plated in duplicate as described

181 previously. The agar plates were incubated at 37 ± 1 °C for 24 h for *Salmonella* and 48

182 h for *L. monocytogenes* and *L. rhamnosus* GG. Each tray was a replicate and there

183 were three replicates of each treatment and each sample date. The experiment was

184 performed twice.

185 To evaluate the results, the populations of the pathogen inoculated alone or in the

186 presence of the antagonist were compared. Reduction of population of foodborne

187 pathogens (FBP) was calculated as follows:

188 Reduction = log N_{FBP} − log $N_{FBP+L.rhamnosus\,GG}$

189 where N_{FBP} is FBP population in the control treatment (FBP alone, CFU g^{-1}) after the

190 storage period and $N_{FBP+\,L.rhamnosus\,GG}$ is FBP population (CFU g^{-1}) after the storage

191 period in the presence of the probiotic.

192

193 2.5. Physicochemical quality

194 Physical and chemical properties of fresh-cut pear were measured on day 0, 2, 6 and

195 9. These analyses were only carried out in treatments a and c.

196

197 2.5.1. Headspace atmosphere

198 Before the microbiological analysis, headspace gas composition (carbon dioxide and

199 oxygen) was determined before opening the trays using a handheld gas analyser

200 (CheckPoint O_2/CO_2, PBI Dansensor, Denmark) at each sampling time. An adhesive

201 septum was stuck on the film and the needle was injected into it to determinate the O_2

202 and CO_2 concentrations. The results are expressed as kPa.

203

204 2.5.2. Measurement of soluble solids contents

205 Soluble solids content (SSC) was measured at 20 °C with a handheld refractometer

206 (Atago Co. Ltd., Tokio, Japan) in juice extracted by crushing pear wedges in a blender.

207 The results are expressed as °Brix.

208

209 2.5.3. Measurement of titratable acidity

210 To measure titratable acidity (TA), 10 mL of pear juice was diluted with 10 mL distilled

211 water and titrated with 0.1 N NaOH up to pH 8.2. The results were calculated as g of

212 malic acid per litre of solution [(mL NaOH × 0.1 N/weight of sample titrated) × 0.067)].

213

214 2.5.4. Determination of headspace ethanol and acetaldehyde concentration

215 Ethanol and acetaldehyde contents were determined according to the protocol

216 described by Echeverría, Graell, López, & Lara (2004). These compounds were

217 extracted from the same juice that was used to determine SSC and TA. Juice samples

218 (5 mL) were stored at -20 °C until analysis. Samples were placed in a 10 mL test tube

219 with a screw cap and incubated in a water bath at 60 °C. After 60 min, a 1 mL

220 headspace gas sample was taken with a syringe and injected into an Agilent

221 Technologies 6890N gas chromatograph for the determination of both acetaldehyde

222 and ethanol concentrations by means of GC. For this purpose, the gas chromatograph

223 was equipped with a flame ionisation detector (FID) and a column (2 m × 2 mm i.d.)

224 containing 5 % Carbowax on 60/80 Carbopack (Supelco, Bellefonte, PA, USA). The

225 temperatures of the injector, detector and oven were 180, 220 and 80 °C, respectively.

226 Tissue concentrations were calculated using ethanol and acetaldehyde calibration

227 curves, undertaken by measuring the headspace of Milli-Q water spiked with a known

228 amount of ethanol and acetaldehyde at increasing concentrations and expressed as μL

229 L^{-1}.

230

231 2.5.5. Determination of volatile compounds

232 Headspace solid phase microextraction (HS-SPME) was used for the extraction and

233 concentration of volatile compounds. SPME fibres coated with a 65 μm thickness of

234 polydimethylsiloxane–divinylbenzene (65 μm PDMS/DVB; Supelco Co., Bellefonte, PA,

235   USA) were used. Fibres were activated before sampling according to the

236   manufacturer's instructions.

237   Four pieces of fruit per tray (n = 3) and treatment were cut into small pieces, frozen

238   with liquid N_2 and crushed, and immediately kept at -80 °C until they were used for the

239   volatile analysis.

240   For each extraction, 4 g of the homogenised crushed pulp was placed into a 20-mL

241   screw-cap vial containing 0.5 g NaCl to facilitate the release of volatile compounds.

242   Prior to sealing the vials, 1μL of 0.086 mg L^{-1} butyl benzene was added as internal

243   standard, and mixed with a glass rod. A magnetic stirrer was added to each vial, which

244   was placed into a constant-temperature water bath at 60 °C for stirring. Samples were

245   equilibrated for 20 min and then the SPME fibre was exposed to the head space of the

246   sample for 30 min in order to adsorb the analytes in accordance with Qin et al. (2012).

247   Volatile compounds were subsequently desorbed for 10 min at 240 °C into the splitless

248   injection port. The volatile constituents were analysed with a HP 5890A gas

249   chromatograph with flame ionisation detector, equipped with a capillary column with

250   cross-linked free fatty acid (FFA) as the stationary phase (FFAP; 50 m $\times$ 0.2 mm $\times$ 0.33

251   μm). A constant column flow of 1.0 mL min^{-1} helium was used as carrier gas. The

252   injector and detector temperatures were 240 °C. The oven temperature program was

253   40 °C for 1 min^{-1}, increased at 2.5 °C min^{-1} to 115 °C, then increased at 8 °C min^{-1} to

254   225 °C and held for 15 min. Compound confirmation was performed in an Agilent

255   6890N gas chromatograph/mass spectrometer (Agilent Technologies, Inc.), using the

256   same capillary column as in the GC analyses. Mass spectra were obtained by electron

257   impact ionisation at 70 eV. Helium was used as the carrier gas, following the same

258   temperature gradient programme as previously described. Spectrometric data were

259   recorded (Hewlett-Packard 3398 GC Chemstation) and compared with data from the

260   original NIST HP59943C library mass spectra. Whenever it was possible, MS

261   identification was confirmed with authentic references. The concentrations of volatile

262   compounds were expressed as ng g^{-1}.

263

264   2.6. Statistical analysis

265   Prior to analysis of variance (ANOVA), all CFU g^{-1} data were transformed to log$_{10}$ CFU

266   g^{-1}. Other data were not transformed. Data were analysed using general linear model

267   analysis with JMP8 software (SAS, 2004).

268   After ANOVA, significant differences between treatments for each sampling time were

269   analysed by using the Student's t test at a significance level of P<0.05.

270   Unscrambler$^{®}$ version 9.1.2. Software (CAMO, 2004) was used to develop a partial

271   least square regression (PLSR) model. The PLSR was used as a predictive method to

272   relate *L. rhamnosus* GG population (Y) to a set of explanatory variables (X) which

273   contained the volatile compound emissions, and O$_2$ and CO$_2$ concentrations. As a pre-

274   treatment, data were centred and weighted using the inverse of the standard deviation

275   of each variable in order to avoid the influence of the different scales used for the

276   variables (Martens & Naes, 1989). Full cross validation was run as a validation

277   procedure.

278

279   3. Results

280

281   3.1. Antagonistic effect of *L. rhamnosus* GG under semi-commercial conditions at 5 °C

282   The results demonstrated that there were no significant differences between the

283   populations of *Salmonella*, *L. monocytogenes* and *L. rhamnosus* GG on fresh-cut pear

284   that were untreated or treated with CaCl$_2$ after harvest. Therefore, the results were

285   pooled.

286   The initial population of *Salmonella* on pear wedges was 3.8 ± 0.1 log$_{10}$ CFU g^{-1} (Fig.

287   1A), regardless of whether it was inoculated alone or with *L. rhamnosus* GG. After 9

288   days of storage at 5 °C, the population remained almost constant. There was no effect

289   of *L. rhamnosus* GG against *Salmonella* since there were no significant differences

290   between fresh-cut pear that were non-inoculated or inoculated with the probiotic strain.

291   In this study, we observed that *L. monocytogenes* grew on pear wedges at a

292   temperature of 5 °C (Fig. 1B). After inoculation, the initial *L. monocytogenes* population

293   was approximately 3.5 ± 0.1 $\log_{10}$ CFU g^{-1} on the fruit non-inoculated or inoculated with

294   *L. rhamnosus* GG. After 2 days of storage, the population of *L. monocytogenes*

295   inoculated without *L. rhamnosus* GG started to increase until it reached 5.8 ± 0.5 $\log_{10}$

296   CFU g^{-1} at the end of the storage. Nevertheless, the population that was co-inoculated

297   with *L. rhamnosus* GG only increased approximately 0.5 log-units after 9 days, which

298   represented 1.8 log-units reduction. No effect was observed after 2 days, but there

299   were significant differences between two treatments after 6 and 9 days of storage.

300

301   3.2. Survival of probiotic strain on fresh-cut pear during storage at 5 °C

302   The initial population of *L. rhamnosus* GG, when it was inoculated alone, was 7.6 ± 0.1

303    $\log_{10}$ CFU g^{-1}, and when it was co-inoculated with the pathogens, it was 7.7 ± 0.2 $\log_{10}$

304   CFU g^{-1}. After 9 days of storage the population of the probiotic remained constant

305   (Figure 2).

306

307   3.3. Physicochemical analysis

308

309   3.3.1. Oxygen and carbon dioxide headspace evolution

310   Inside the trays, the O_2 concentration dropped rapidly from 21.0 kPa to approximately

311   10.0 kPa after 2 days of storage, reaching values of 0.0 kPa after 6 days of storage,

312   and remaining at this concentration until the end of the study. There were no significant

313   differences between pears untreated and treated with *L. rhamnosus* GG. The CO_2

314   concentration increased quickly to 19.5 kPa in pear wedges untreated with the probiotic

315   and approximately 22.0 kPa in pear wedges treated with *L. rhamnosus* GG after 9 days

316   of storage with significant differences between treatments with this storage time (Fig.

317   3).

318

319   3.3.2. Soluble solids content and titratable acidity

320   Soluble solids content (SSC) and total titrable acidity (TA) of pear wedges were

321   determined in control and *L. rhamnosus* GG treatments. Initial values of SSC ranged

322   from 13.4 ± 0.1 to 13.1 ± 0.1 °Brix and during the assay they had negligible variance

323   (data not shown). For TA, initial values ranged between 1.68 ± 0.04 and 2.09 ± 0.05 g

324   malic acid L^{-1} and they remained almost constant during the storage and did not follow

325   any trend (data not shown).

326

327   3.3.3. Ethanol and acetaldehyde concentration

328   There were no significant differences in ethanol and acetaldehyde concentration

329   between pears untreated or treated with $CaCl_2$ after the harvest, therefore data were

330   pooled.

331   Initial concentration of ethanol was approximately 109 µL L^{-1} for pear wedges untreated

332   and 77 µL L^{-1} for pear wedges treated with *L. rhamnosus* GG with no significant

333   differences (Fig. 4A). Ethanol concentration increased throughout the storage by up to

334   approximately 600 µL L^{-1} in pear wedges untreated and 740 µL L^{-1} in pear wedges

335   treated with the probiotic after 9 days of storage at 5 °C. No significant differences were

336   noted among the treatments.

337   Initial acetaldehyde concentration was approximately 7 and 6 µL L^{-1} in untreated and *L.*

338   *rhamnosus* GG in treated pear wedges (Fig. 4B). After 9 days of storage the

339   concentration raised to 18 µL L^{-1} in pear wedges untreated and to 21 µL L^{-1} in pears

340   treated with the probiotic. No significant differences between two treatments were

341   observed.

342

343   3.3.4. Relationships between samples and volatile compound emissions

344   The effects of the probiotic inclusion on the volatile compounds emitted by pear

345   wedges were investigated. A total of 48 compounds (27 esters, 10 alcohols, 4

346   aldehydes, 2 terpenes, 2 ketones and 1 acid) were identified and quantified in the

13

347 volatile fraction emitted by fruit (data not shown). A partial least square regression

348 model (PLSR) was developed, with the aim of assessing possible correlations between

349 *L. rhamnosus* GG population (*Y variable*) and a set of potentially explanatory variables

350 (*X variables*) which included the concentration of the volatile compounds emitted by

351 pear wedges. In order to refine differentiation between the control and *L. rhamnosus*

352 GG-treated samples, samples from day 0 (treatment time) were excluded from the

353 analysis. Consequently, a PLSR was performed, which include 12 samples and 59

354 variables (48 volatile compounds and the total emission of the different families of

355 acetates, propanoates, butanoates, pentanoates, hexanoates, octanoates, alcohols,

356 aldehydes, terpenes, ketones and acids) (Fig. 5). The validation step indicates that two

357 PLS factors are relevant in the model. According to this model, up to 70 % of variability

358 in the samples is explained by the volatile compounds emissions (Fig. 5). *L. rhamnosus*

359 GG-treated samples are located more on the right side of the PC1, which alone explain

360 56 % of total variance, if compared to control fruit located on the left side of the PC1

361 (Fig. 5A). The corresponding loadings plot (Fig. 5B) shows that these samples that

362 were treated with *L. rhamnosus* GG are associated with higher concentrations of some

363 alcohol and ester characteristics in the volatile profile of pears than in the control

364 samples, which could indicate a better conservation of the typical flavour of this pear

365 cultivar compared to control samples. No clear influence of volatile compounds on the

366 differentiation of calcium and non-calcium treated samples, as well as on the days of

367 storage at 5 °C after processing was observed.

368 Figure 6 shows the regression coefficients for *L. rhamnosus* GG population vs. the

369 volatile compound emissions. This figure permits us to identify those volatiles that have

370 the most influence on the *L. rhamnosus* GG population. It can be seen that the *L.*

371 *rhamnosus* GG population was positively related to higher emissions of certain esters

372 (methyl acetate, propyl acetate, hexyl acetate, (E)-2-hexenyl acetate, ethyl 2-

373 methylbutanoate, 2-methylbutyl 2-methylbutanoate and pentyl 3-methylbutanoate),

374 some alcohols (ethanol, 3-methyl-2-butanol, 1-hexanol and benzyl alcohol) and one

375 aldehyde (benzaldehyde).

376

377 4. Discussion

378 To the best of our knowledge, there are only a few studies that have been realised

379 concerning biocontrol of foodborne pathogens on fresh-cut fruit using probiotic bacteria

380 (Alegre et al., 2011; Russo et al., 2014; 2015). Moreover, the positive effect of fruit

381 intake and a regular consumption of viable probiotics on some cancers and

382 cardiovascular diseases has been widely reported (Cross, 2002; McCann et al., 2007;

383 Nguyen, Kang, & Lee, 2007). This has created a growing interest in fruit products that

384 are enriched with these types of components (Rößle et al., 2010b). However, to the

385 best of our knowledge, only a few studies where the influence on pear quality, or more

386 specifically on pear flavor, due to their combination have been reported. The present

387 study was focused on the control of foodborne pathogens on fresh-cut pear using a

388 probiotic strain and was aimed at determining its effect on several quality aspects,

389 including volatile compounds.

390 We have confirmed that *L. rhamnosus* GG controlled growth of *L. monocytogenes* and

391 survived during storage at 5 °C in modified atmosphere. Moreover, we did not observe

392 significant differences in SSC between *L. rhamnosus* GG-treated and untreated pears.

393 Similar results were reported on apple (Alegre et al. 2011; Rößle, Auty, Brunton,

394 Gormley, & Butler, 2010a) and melon minimally processed (Oliveira, Leite, Martins,

395 Martins, & Ramos, 2014). We also did not observe significant differences in TA values

396 between two treatments and throughout the storage in accordance with Rößle et al.

397 (2010a) in apple. However, Alegre et al. (2011) found significant differences in TA in

398 apple after 7 days of storage at 10 °C. The application of *L. rhamnosus* GG did not

399 affect the quality (SSC and TA) of the fresh-cut pear after 9 days of storage. Similarly,

400 Russo et al. (2015) and Siroli et al. (2015) demonstrated that a high dose of probiotic

401　did not affect most of the sensory qualities after 11 days of storage in fresh-cut melon

402　and in apple wedges after 9 days of storage, respectively.

403　It is known that in the presence of low O_2 and high CO_2 concentrations, *L. rhamnosus*

404　GG can ferment sugars as glucose, fructose, lactose and sucrose producing several

405　metabolites including ethanol and acetaldehyde (Hedberg, Hasslöf, Sjöström,

406　Twetman, & Stecksén-Blicks, 2008). Moreover, low O_2 concentration inside the trays

407　could activate ethanolic fermentation in fruit tissues and, as a consequence,

408　acetaldehyde and ethanol are released. Acetaldehyde is converted into ethanol by the

409　enzyme alcohol dehydrogenase, hence the final product of this ethanolic fermentation

410　pathway is ethanol (Ke, Yahia, Mateos, & Kader, 1994). We observed that the

411　concentration of both metabolites increased thoroughout the assay, but we did not find

412　significant differences between pear wedges that were untreated and treated with *L.*

413　*rhamnosus* GG. This finding could indicate that ethanol and acetaldehyde were

414　products of fermentation pathways in fruit tissues and they did not come from

415　fermentation reactions produced by the probiotic.

416　More than 100 volatile compounds have been identified in pear, including aldehydes,

417　alcohols, esters and ketones (Qin et al., 2012). Among them, volatile esters, for

418　example, butyl acetate, (Z)-3-hexenyl acetate, amyl acetate, isobutyl acetate, hexyl

419　acetate, butyl propionate, (E)-2-hexenyl acetate are the main contributors to pear odour

420　(Aprea et al., 2012). The 2-methylpropyl acetate was the main ester produced by

421　'Conference' pear after 5 months in CA storage with "sharp" odour notes and the

422　volatile compounds butanol and ethyl butanoate were considered responsible for a

423　"ripe pear" aroma (Rizzolo, Cambiaghi, Grassi, & Zerbini, 2005). From the 48 volatile

424　compounds identified, using a partial least square regression model, we could detect

425　12 compounds that were key variables for the discrimination of the samples in two

426　groups (control and *L. rhamnosus* GG-treated samples). These were: methyl acetate,

427　propyl acetate, hexyl acetate, (E)-2-hexenyl acetate, ethyl 2-methylbutanoate, 2-

428　methylbutyl 2-methylbutanoate, pentyl 3-methylbutanoate, ethanol, 3-methyl-2-butanol,

429   1-hexanol, benzyl alcohol and benzaldehyde. The higher concentration of these 12

430   volatiles that were detected in *L. rhamnosus* GG-treated samples should be

431   understood in conjunction with sensorial descriptors. From a sensory point of view, the

432   positive or negative effect of a volatile is mainly due to their quantitative abundance,

433   olfactory thresholds and, of course, to the odour descriptor (Schieberle, Ofner, &

434   Grosch, 1990; Wyllie, Leach, Wang, & Shewfelt, 1995). The odour descriptors of these

435   twelve volatile compounds are: pear-raspberry (propyl acetate), fruity (hexyl acetate

436   and methyl acetate), powerful and fresh-green, sweet and fruity ((E)-2-hexenyl

437   acetate), ripe apple (ethyl 2-methylbutanoate), fruity (2-methylbutyl 2-methylbutanoate),

438   apple fresh fruity (pentyl 3-methylbutanoate), sweet (ethanol), sweet, fruit, floral, fig,

439   rose and nutty (3-methyl-2-butanol), herbal, fatty and fruity aroma (1-hexanol), sun

440   flower seeds, herbal and mouldy (benzyl alcohol) and bitter almond and fresh plum

441   aroma (benzaldehyde). Considering these descriptors, we can highlight that the

442   detected increase in these volatile compounds in *L. rhamnosus* GG-treated samples

443   will be positive in the flavour perception of these samples by consumers. Similarly,

444   Rößle et al. (2010a) found that probiotic *L. rhamnosus* GG apples had a high amount of

445   hexyl acetate. In relation to the higher acetate ester emission detected in *L. rhamnosus*

446   GG-treated samples, we are not able to determine if it was due to the interaction of fruit

447   with the probiotic bacteria or to its own production of acetate esters. Aroma and flavour

448   VCs, including esters, aldehydes and alcohols produced by bacteria, yeasts and fungi

449   have been detected (Alves Macedo, Alves Macedo, & Francisco Fleuri, 2010; Amaral,

450   Rocha-Leao, & Coelho, 2010; Pandey, Soccol, & Mitchell, 2000).

451   To summarise, *L. rhamnosus* GG was able to control the growth of *L. monocytogenes*

452   on fresh-cut pear, without affecting fruit quality. The present study shows that VCs

453   detected in fresh-cut pear treated with the probiotic could add good flavour to the

454   product. The population of *L. rhamnosus* GG remained constant on pear wedges

455   during storage, which could suggest that MP pear is a good vehicle for carrier probiotic

456   microorganisms for people who do not have another source of probiotic, such as

457   vegetarians or those who are lactose intolerant. It would be very interesting to add a

458   consumer's test in future research in order to assess whether this increase would have

459   a positive effect on consumer satisfaction.

460

461   Acknowledgments

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Figure legends

Figure 1. Population (log CFU g^{-1}) of *Salmonella* (A) and *L. monocytogenes* (B) inoculated alone (continuous line) or co-inoculated with *L. rhamnosus* GG (dotted line) on fresh-cut 'Conference' pear wedges stored at 5 °C. Results are mean of twelve values and vertical bar indicates the standard deviation of the mean. For each storage time, different letters indicate significant differences among treatments according to a Student's t-test (P < 0.05).

Figure 2. Enumeration of *L. rhamnosus* GG (log CFU g^{-1}) inoculated alone (continuous line) or co-inoculated with pathogens (dotted line) on fresh-cut 'Conference' pear wedges stored at 5 °C. Results are mean of twelve values and vertical bar indicates the standard deviation of the mean. For each storage time, different letters indicate significant differences between treatments according to a Student's t-test (P < 0.05).

Figure 3. O_2 and CO_2 headspace concentration (kPa) inside pear trays treated with *L. rhamnosus* GG (black) and pear trays untreated with *L. rhamnosus* GG (grey) throughout storage time at 5 °C. Results are the average of twelve values. Vertical bars indicate the standard deviation of the mean.

* Indicates significant differences between treatments for each storage time (P < 0.05).

Figure 4. Concentration (µL L^{-1}) of ethanol (A) and acetaldehyde (B) produced on pear wedges stored at 5 °C in presence of *L. rhamnosus* GG (grey) or without *L. rhamnosus* GG. Results are the mean of 6 values. Different letters indicate significant differences among treatments according to Student's t-test (P < 0.05).

Figure 5. Scores (A) and loadings (B) plot of PC1 vs. PC2 corresponding to a PLSR model for *L. rhamnosus* GG population vs. emissions of volatile compounds.

Figure 6. Regression coefficients corresponding to a PLSR model for *L. rhamnosus* GG population vs. emissions of volatile compounds.
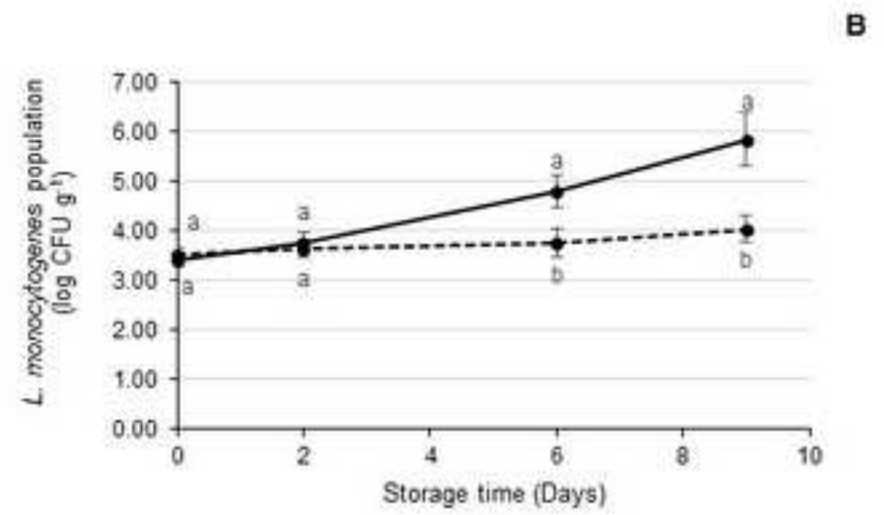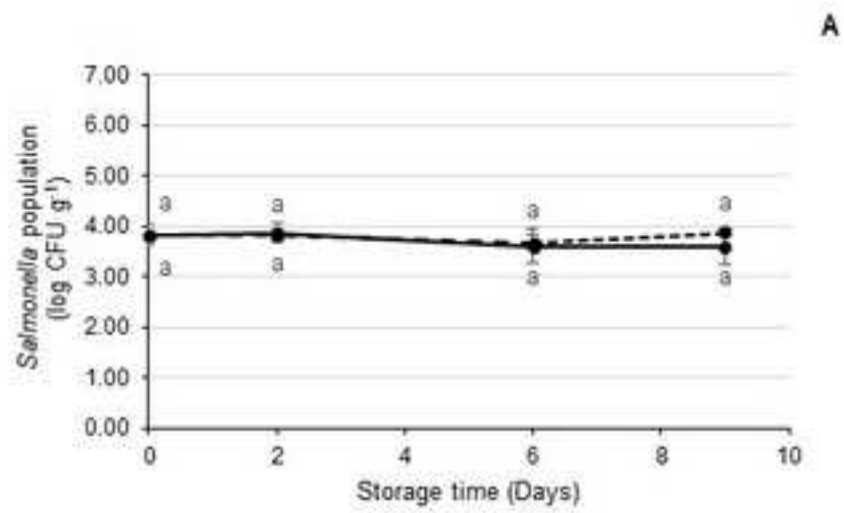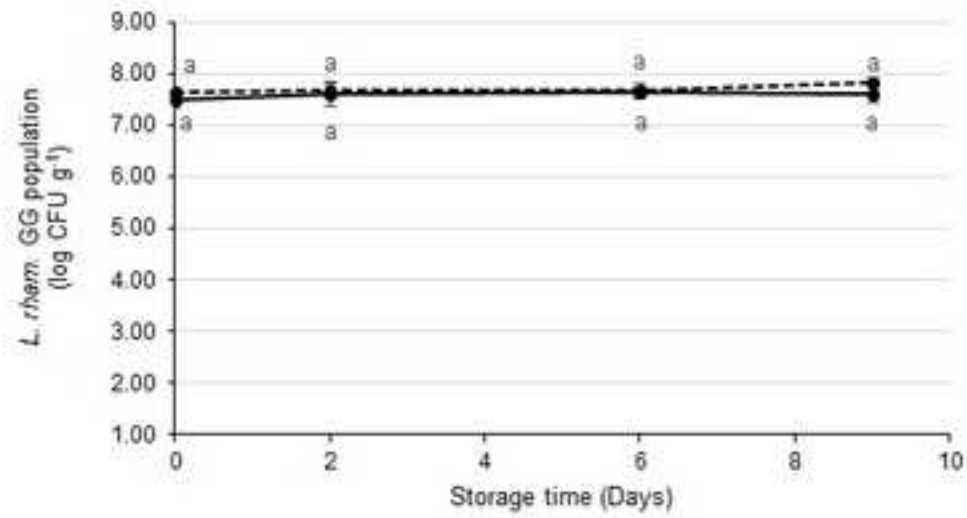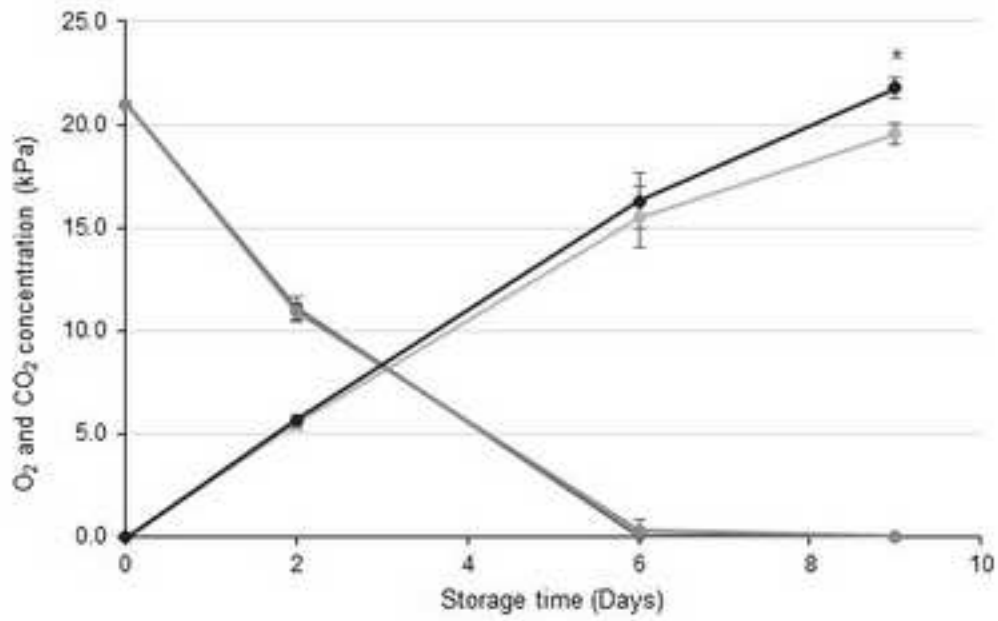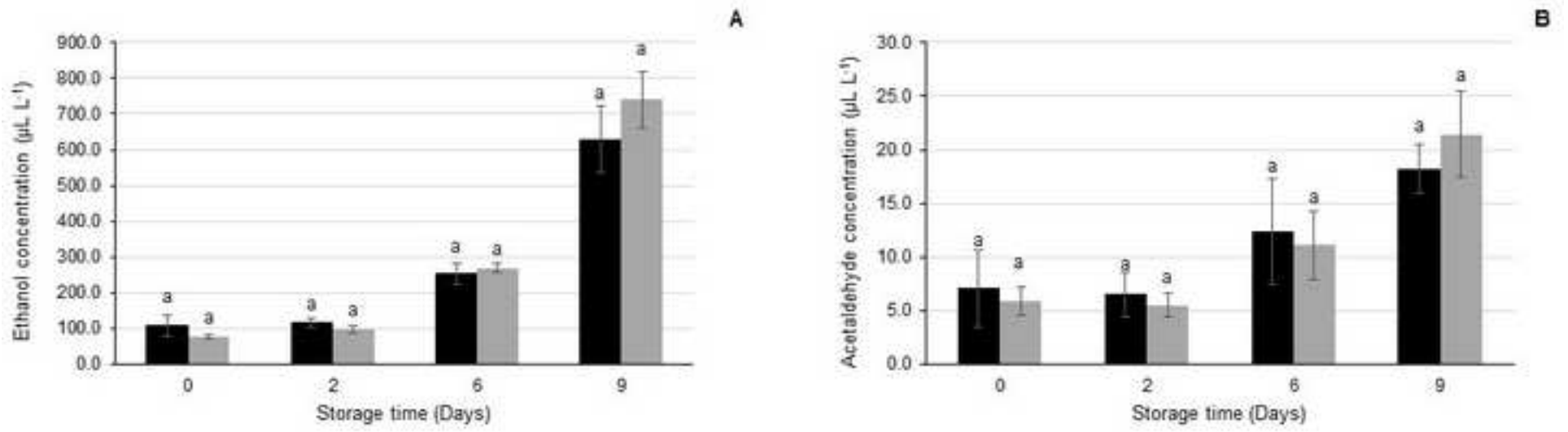
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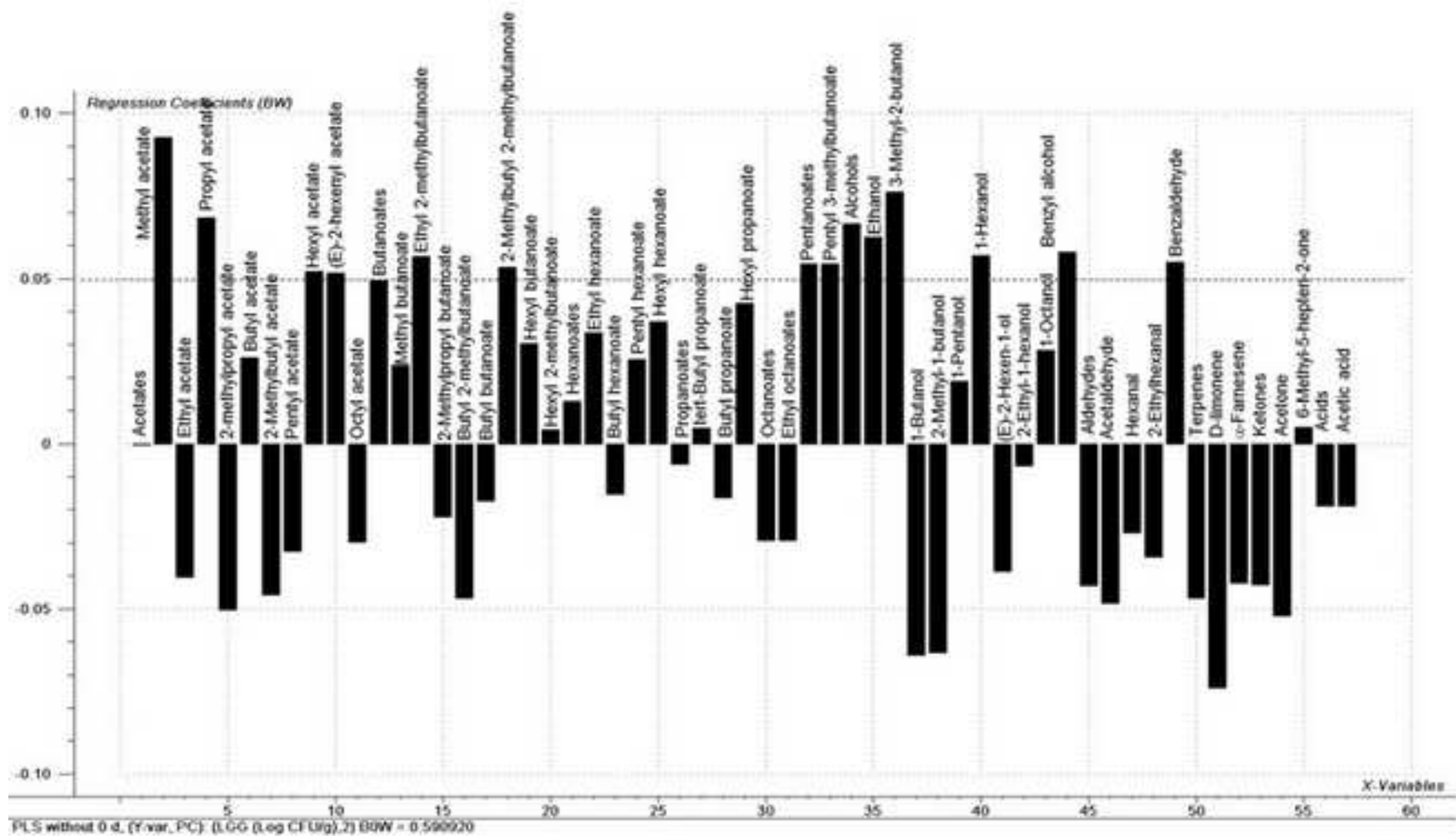
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Lleida, 29th June 2017

Dear all,

(Team from LWT - Food Science and Technology)

I am writing to you regarding the manuscript entitled "Biopreservation of fresh-cut pear using *Lactobacillus rhamnosus* GG and effect on quality and volatile compounds", which has been sent back to the corresponding author before the scientific assessment.

We are aware that research papers sent to LWT should not exceed 5500 words. We have reviewed the manuscript again and made some changes, but our manuscript still has 6292 words (including abstract and references but excluding figures, tables and their captions).

The work presented in this manuscript is a complete study of the effectiveness of a probiotic strain against foodborne pathogens and its effect on fruit quality, detailing the volatile compounds profile. To the best of our knowledge, it represents novel work, which has not been studied before. From our point of view, if we further reduce the length of the paper and take out more references, the quality of the manuscript will be compromised. We believe that all the data and information provided is relevant and we ask you if the submission process could continue even if we exceed the limit (by 700 words, 13%). We think that the work presented fits well with the scope of your journal. If not, we unfortunately would reconsider the submission and submit the paper to another journal.

Thank you very much in advance and sorry for any inconvenience.

If you need any more information, please do not hesitate to contact me.

Yours sincerely,

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