Biopreservation of fresh-cut pear using *Lactobacillus rhamnosus* GG and effect on quality and volatile compounds

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Abstract

In recent years, the consumption of minimally processed fruit has increased. However, unfortunately, these products could be an appropriate vehicle for the transmission of foodborne pathogens. In this study, the antagonistic capacity of the probiotic strain *Lactobacillus rhamnosus* GG against a cocktail of 5 serovars of *Salmonella* and 5 serovars of *Listeria monocytogenes* on fresh-cut pear at conditions simulating commercial application was assessed. Moreover, its effect on fruit quality, particularly on the volatile profile, was determined, during 9 days of storage at 5 °C. *L. mo*nocytogenes population was reduced by approximately 1.8 log-units when co-inoculated with *L. rhamnosus* GG. However, no effect was observed in *Salmonella*. Fruit quality (soluble solids content and titratable acidity) did not change when the probiotic was present. A total of 48 volatile compounds were identified using gas chromatography. Twelve of the compounds allowed to discriminate *L. rhamnosus* GG-treated and untreated pears. Considering their odour descriptors, their increases could be positive in the flavour perception of *L. rhamnosus* GG-treated pear. The probiotic was able to control *L. monocytogenes* population on fresh-cut pear, which could be a vehicle of probiotic microorganisms as quality of fruit was not affected when the probiotic was present.

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

1. Introduction

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

fact that they are easy to consume, and also fresh and healthy because of their nutritional contribution (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). Fresh fruits are generally considered to be microbiologically safe. However, they could be contaminated in the preharvest environment due to the irrigation water, air, compost, animals, human handling … and also during harvest and postharvest (Beuchat, 1995). Moreover, when fruit is processed, bacteria may be transferred from external fruit surfaces to edible portions, being a potential vehicle for the transmission of the main foodborne pathogens such as *Salmonella*, *Escherichia coli* or *Listeria monocytogenes* (Ukuku, Geveke, Chau, & Niemira, 2016). *L. monocytogenes* is able to grow at refrigerated temperature on fresh cut apple (Alegre, Viñas, Usall, Anguera, & Abadias, 2011), melon (Abadias et al., 2014) and on melon, apple and mango at 7 °C (Lokerse, Maslowska-Corker, van de Wardt, & Wijtzes, 2016). Moreover, controlled atmosphere storage does not appear to influence growth rates (Oliveira, Abadias, Colas-Meda, Usall, & Viñas, 2015).

In order to reduce pathogenic microorganisms, different techniques have been studied, one of which is biopreservation using lactic acid bacteria (LAB). LAB are able to convert lactose and other sugars in lactic acid and could generate other final metabolites such as ethanol if they carry out a heterolactic fermentation (Li, 2004). Another characteristic of this genus is that most of the bacteria which are included in it are considered to be probiotics. According to reports by FAO/WHO (2002), probiotics are defined as living microorganisms that, when administered in adequate amounts, confer benefits to host health, through a positive action of intestinal microbiota. The way in which probiotics provide beneficial effects on health is, mainly, by activating the immune system, improving intestinal microbial balance and controlling foodborne pathogens. Some LAB also have antimicrobial activity, which is carried out by secreting antimicrobial byproducts, such as lactic acid, hydrogen peroxide and polypeptides, inhibiting or blocking adhesion to epithelial cells and the invasion abilities of enteropathogens (Ng, Hart, Kamm, Stagg, & Knight, 2009; Peng, Reichmann, &

Biswas, 2015). Some probiotic bacteria have demonstrated a good ability to reduce the level of foodborne pathogens on fresh-cut fruit. Russo et al. (2014; 2015) demonstrated that some probiotic strains have an antagonistic effect against *L. monocytogenes* on fresh-cut pineapple and melon and Siroli et al. (2015) demonstrated the same effect on fresh-cut apples. *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG) demonstrated to have a bacteriostatic effect against *L. monocytogenes* and *Salmonella* on fresh-cut apple (Alegre et al., 2011) and pear (Iglesias, Abadias, Anguera, Sabata, & Viñas, 2017). However, little is known about the effect of the application of this probiotic strain on the quality of fresh-cut fruit and, in particular, on the volatile compounds (VCs) (Rößle, Brunton, Gormley, Ross, & Butler, 2010b).

Salmeron, Loeza-Serrano, Perez-Vega, & Pandiella (2015) studied VCs produced by three different lactobacilli in barley and malt fermentation and they observed that the VC profile varies, depending on the matrix. The VC profile can also provide desirable sensorial notes for the consumer, contributing to the characteristic flavour and aroma in determinate foods (Sreekumar, Al-Attabi, Deeth, & Turner, 2009). In the case of lactobacilli fermentations, VCs such as ethanol, acetaldehyde, acetone, diacetyl, and ethyl acetate are produced and which are responsible for the flavour in specific foods and beverages (Beshkova, Simova, Frengova, Simov, & Dimitrov, 2003; Salmeron et al., 2015). Nevertheless, the same VCs could cause off-flavour notes and non-pleasant flavours in some matrix food (Kopsahelis, Kanellaki, & Bekatorou, 2007). It is important to know about the evolution of quality attributes of fresh-cut products, such as odour, taste, colour and texture in order to relate with microbiological and physiological features during the product storage.

The combination of probiotic strains with fruit could be promising due to the fact that it could be one way to help vegetarians, vegans and people who are allergic to dairy food to ingest these bacteria from alternative sources and obtain their benefits (Luckow & Delahunty, 2004).

The aim of this study was to evaluate the effect of the application of *L. rhamnosus* GG on the quality of fresh-cut pear at conditions simulating commercial application with special emphasis on the volatile compounds. Pears were treated or not-treated with $CaCl_2$ after harvest and stored in controlled atmosphere (CA) conditions before processing. The antagonistic effect of *L. rhamnosus* GG against *L. monocytogenes* and *Salmonella* was validated. To the best of our knowledge, this study is the first to evaluate sensorial aspects of fresh-cut pear treated with a probiotic strain simulating commercial conditions.

2. Material and methods

2.1. Fruit

'Conference' pears (*Pyrus communis* L. cv. Conference) were used in this experiment. After harvest, pears were divided into two lots. Whole fruits of lot 1 were dipped in water at 25 °C for 5 min and this group was used as control. Whole fruits of lot 2 were dipped in a solution containing 10 g L^{-1} $CaCl_2$ at 25 °C during 5 min. After fruit harvest, cold storage and CA are essential to delay the ripening process. In apples, postharvest dipping in $CaCl_2$ before storage contribute to extending commercial life in whole fruit as well as minimally processed (MP) fruit.

Afterwards, pears of both lots were stored at 0 ± 1 °C during 8 months in CA (2 kPa O_2 and 1 kPa CO_2) up to the time of the experiment. After this storage time, the pears were conditioned at 20 °C until the optimum ripeness stage for processing (44 ± 3.2 N) (Soliva-Fortuny, Alos-Saiz, Espachs-Barroso, & Martin-Belloso, 2004).

2.2. Bacterial strains and inoculum preparation

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

(SP; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone) and plated in duplicate as described previously. The agar plates were incubated at 37 ± 1 °C for 24 h for *Salmonella* and 48 h for *L. monocytogenes* and *L. rhamnosus* GG. Each tray was a replicate and there were three replicates of each treatment and each sample date. The experiment was performed twice.

To evaluate the results, the populations of the pathogen inoculated alone or in the presence of the antagonist were compared. Reduction of population of foodborne pathogens (FBP) was calculated as follows:

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

where N_{FBP} is FBP population in the control treatment (FBP alone, CFU g^{-1}) after the storage period and $N_{FBP+\ L.rhamnosus\ GG}$ is FBP population (CFU g^{-1}) after the storage period in the presence of the probiotic.

2.5. Physicochemical quality

Physical and chemical properties of fresh-cut pear were measured on day 0, 2, 6 and 9. These analyses were only carried out in treatments a and c.

2.5.1. Headspace atmosphere

Before the microbiological analysis, headspace gas composition (carbon dioxide and oxygen) was determined before opening the trays using a handheld gas analyser (CheckPoint O_2/CO_2, PBI Dansensor, Denmark) at each sampling time. An adhesive septum was stuck on the film and the needle was injected into it to determinate the O_2 and CO_2 concentrations. The results are expressed as kPa.

2.5.2. Measurement of soluble solids contents

Soluble solids content (SSC) was measured at 20 °C with a handheld refractometer (Atago Co. Ltd., Tokio, Japan) in juice extracted by crushing pear wedges in a blender. 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,

USA) were used. Fibres were activated before sampling according to the manufacturer's instructions.

Four pieces of fruit per tray (n = 3) and treatment were cut into small pieces, frozen with liquid N_2 and crushed, and immediately kept at -80 °C until they were used for the volatile analysis.

For each extraction, 4 g of the homogenised crushed pulp was placed into a 20-mL screw-cap vial containing 0.5 g NaCl to facilitate the release of volatile compounds. Prior to sealing the vials, 1µL of 0.086 mg L^{-1} butyl benzene was added as internal standard, and mixed with a glass rod. A magnetic stirrer was added to each vial, which was placed into a constant-temperature water bath at 60 °C for stirring. Samples were equilibrated for 20 min and then the SPME fibre was exposed to the head space of the sample for 30 min in order to adsorb the analytes in accordance with Qin et al. (2012). Volatile compounds were subsequently desorbed for 10 min at 240 °C into the splitless injection port. The volatile constituents were analysed with a HP 5890A gas chromatograph with flame ionisation detector, equipped with a capillary column with cross-linked free fatty acid (FFA) as the stationary phase (FFAP; 50 m $\times$ 0.2 mm $\times$ 0.33 µm). A constant column flow of 1.0 mL min^{-1} helium was used as carrier gas. The injector and detector temperatures were 240 °C. The oven temperature program was 40 °C for 1 min^{-1}, increased at 2.5 °C min^{-1} to 115 °C, then increased at 8 °C min^{-1} to 225 °C and held for 15 min. Compound confirmation was performed in an Agilent 6890N gas chromatograph/mass spectrometer (Agilent Technologies, Inc.), using the same capillary column as in the GC analyses. Mass spectra were obtained by electron impact ionisation at 70 eV. Helium was used as the carrier gas, following the same temperature gradient programme as previously described. Spectrometric data were recorded (Hewlett-Packard 3398 GC Chemstation) and compared with data from the original NIST HP59943C library mass spectra. Whenever it was possible, MS identification was confirmed with authentic references. The concentrations of volatile 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.

In this study, we observed that *L. monocytogenes* grew on pear wedges at a temperature of 5 °C (Fig. 1B). After inoculation, the initial *L. monocytogenes* population was approximately 3.5 ± 0.1 $\log_{10}$ CFU g^{-1} on the fruit non-inoculated or inoculated with *L. rhamnosus* GG. After 2 days of storage, the population of *L. monocytogenes* inoculated without *L. rhamnosus* GG started to increase until it reached 5.8 ± 0.5 $\log_{10}$ CFU g^{-1} at the end of the storage. Nevertheless, the population that was co-inoculated with *L. rhamnosus* GG only increased approximately 0.5 log-units after 9 days, which represented 1.8 log-units reduction. No effect was observed after 2 days, but there were significant differences between two treatments after 6 and 9 days of storage.

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

The initial population of *L. rhamnosus* GG, when it was inoculated alone, was 7.6 ± 0.1 $\log_{10}$ CFU g^{-1}, and when it was co-inoculated with the pathogens, it was 7.7 ± 0.2 $\log_{10}$ CFU g^{-1}. After 9 days of storage the population of the probiotic remained constant (Figure 2).

3.3. Physicochemical analysis

3.3.1. Oxygen and carbon dioxide headspace evolution

Inside the trays, the O_2 concentration dropped rapidly from 21.0 kPa to approximately 10.0 kPa after 2 days of storage, reaching values of 0.0 kPa after 6 days of storage, and remaining at this concentration until the end of the study. There were no significant differences between pears untreated and treated with *L. rhamnosus* GG. The CO_2 concentration increased quickly to 19.5 kPa in pear wedges untreated with the probiotic and approximately 22.0 kPa in pear wedges treated with *L. rhamnosus* GG after 9 days of storage with significant differences between treatments with this storage time (Fig. 3).

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

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,

1-hexanol, benzyl alcohol and benzaldehyde. The higher concentration of these 12 volatiles that were detected in *L. rhamnosus* GG-treated samples should be understood in conjunction with sensorial descriptors. From a sensory point of view, the positive or negative effect of a volatile is mainly due to their quantitative abundance, olfactory thresholds and, of course, to the odour descriptor (Schieberle, Ofner, & Grosch, 1990; Wyllie, Leach, Wang, & Shewfelt, 1995). The odour descriptors of these twelve volatile compounds are: pear-raspberry (propyl acetate), fruity (hexyl acetate and methyl acetate), powerful and fresh-green, sweet and fruity ((E)-2-hexenyl acetate), ripe apple (ethyl 2-methylbutanoate), fruity (2-methylbutyl 2-methylbutanoate), apple fresh fruity (pentyl 3-methylbutanoate), sweet (ethanol), sweet, fruit, floral, fig, rose and nutty (3-methyl-2-butanol), herbal, fatty and fruity aroma (1-hexanol), sun flower seeds, herbal and mouldy (benzyl alcohol) and bitter almond and fresh plum aroma (benzaldehyde). Considering these descriptors, we can highlight that the detected increase in these volatile compounds in *L. rhamnosus* GG-treated samples will be positive in the flavour perception of these samples by consumers. Similarly, Rößle et al. (2010a) found that probiotic *L. rhamnosus* GG apples had a high amount of hexyl acetate. In relation to the higher acetate ester emission detected in *L. rhamnosus* GG-treated samples, we are not able to determine if it was due to the interaction of fruit with the probiotic bacteria or to its own production of acetate esters. Aroma and flavour VCs, including esters, aldehydes and alcohols produced by bacteria, yeasts and fungi have been detected (Alves Macedo, Alves Macedo, & Francisco Fleuri, 2010; Amaral, Rocha-Leao, & Coelho, 2010; Pandey, Soccol, & Mitchell, 2000).

To summarise, *L. rhamnosus* GG was able to control the growth of *L. monocytogenes* on fresh-cut pear, without affecting fruit quality. The present study shows that VCs detected in fresh-cut pear treated with the probiotic could add good flavour to the product. The population of *L. rhamnosus* GG remained constant on pear wedges during storage, which could suggest that MP pear is a good vehicle for carrier probiotic 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
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471 References
472 Abadias, M., Altisent, R., Usall, J., Torres, R., Oliveira, M., & Viñas, I. (2014).
473 Biopreservation of fresh-cut melon using the strain Pseudomonas graminis
474 CPA-7. *Postharvest Biology and Technology*, 96, 69-77.

475 Abadias, M., Usall, J., Anguera, M., Solsona, C., & Viñas, I. (2008). Microbiological
476 quality of fresh, minimally-processed fruit and vegetables, and sprouts from
477 retail establishments. *International Journal of Food Microbiology, 123*(1-2), 121-
478 129.

479 Alegre, I., Viñas, I., Usall, J., Anguera, M., & Abadias, M. (2011). Microbiological and
480 physicochemical quality of fresh-cut apple enriched with the probiotic strain
481 *Lactobacillus rhamnosus* GG. *Food Microbiology, 28*(1), 59-66.

Alves, G., Alves, J., & Francisco, L. (2010). Fermentation and fruit flavor production. In Y. H. Hui (Ed.), *Handbook of Fruit and Vegetable Flavors.* (pp. 59-72). Hoboken, NY: John Wiley & Sons, Inc.

Amaral, P. F. F., Rocha-Leao, M. H. M., & Coelho, M. A. Z. (2010). Bioconversion of flavors. In Y. H. Hui (Ed.), *Handbook of Fruit and Vegetable Flavors* (pp. 115-128). Hoboken, NY: John Wiley & Sons, Inc.

Aprea, E., Corollaro, M. L., Betta, E., Endrizzi, I., Demattè, M. L., Biasioli, F., & Gasperi, F. (2012). Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. *Food Research International, 49*(2), 677-686.

Beshkova, D. M., Simova, E. D., Frengova, G. I., Simov, Z. I., & Dimitrov, Z. P. (2003). Production of volatile aroma compounds by kefir starter cultures. *International Dairy Journal, 13*(7), 529-535.

Beuchat, L. R. (1995). Pathogenic Microorganisms Associated with Fresh Produce. *Journal of Food Protection*, *59*(2), 204–216.

CAMO ASA, Unscramblers User Guide, ver. 9.1.2. Programme Package for Multivariate Calibration. Trondheim, Norway. (2004).

Cross, M. L. (2002). Immunoregulation by probiotic lactobacilli. *Clinical and Applied Immunology Reviews, 3*(3), 115-125.

de Oliveira, P. M., Leite, B. R., Martins, M. L., Martins, E. M. F., & Ramos, A. M. (2014). Minimally processed yellow melon enriched with probiotic bacteria. *Semina-Ciencias Agrarias, 35*(5), 2415-2425.

Echeverría, G., Graell, J., López, M. L., & Lara, I. (2004). Volatile production, quality and aroma-related enzyme activities during maturation of 'Fuji' apples. *Postharvest Biology and Technology, 31*(3), 217-227.

FAO/WHO. (2002). Guidelines for the Evaluation of Probiotics in Food. Food and Agriculture Organization of the United Nations and World Health Organization. Working Group Report.

510 Hedberg, M., Hasslöf, P., Sjöström, I., Twetman, S., & Stecksén-Blicks, C. (2008).
511 Sugar fermentation in probiotic bacteria – an *in vitro* study. *Oral Microbiology*
512 *and Immunology, 23*(6), 482-485.
513 Iglesias, M. B., Abadias, M., Anguera, M., Sabata, J., & Viñas, I. (2017). Antagonistic
514 effect of probiotic bacteria against foodborne pathogens on fresh-cut pear. *LWT*
515 *- Food Science and Technology, 81*, 243-249.
516 Ke, D. Y., Yahia, E., Mateos, M., & Kader, A. A. (1994). Ethanolic fermentation of
517 bartlett pears as influenced by ripening stage and atmospheric composition.
518 *Journal of the American Society for Horticultural Science, 119*(5), 976-982.
519 Kopsahelis, N., Kanellaki, M., & Bekatorou, A. (2007). Low temperature brewing using
520 cells immobilized on brewer's spent grains. *Food Chemistry, 104*(2), 480-488.
521 Li, K.Y. (2004), Fermentation: principles and microorganisms. In Y. H. Hui, S. Ghazala,
522 D. M. Graham, K. D. Murrell & W. K. Nip (Eds.), *Handbook of Vegetable*
523 *Preservation and Processing.* (pp. 612-626). New York, NY: Marcel Dekker, Inc.
524 Lokerse, R. F. A., Maslowska-Corker, K. A., van de Wardt, L. C., & Wijtzes, T. (2016).
525 Growth capacity of Listeriamonocytogenes in ingredients of ready-to-eat salads.
526 *Food Control*, *60*, 338–345.
527 Luckow, T., & Delahunty, C. (2004). Which juice is 'healthier'? A consumer study of
528 probiotic non-dairy juice drinks. *Food Quality and Preference, 15*(7-8), 751-759.
529 Martens H., & Naes T.. (1989). *Partial least squares regression. Multivariate*
530 *calibration*. Chichester: J. Wiley & Sons, Inc.
531 McCann, M. J.,Gill, C. I., O' Brien G., Rao, J. R., McRoberts, W. C., Hughes, P.,
532 McEntee, R., Rowland, I. R. (2007). Anti-cancer properties of phenolics from
533 apple waste on colon carcinogenesis *in vitro*. *Food and Chemical Toxicology,*
534 *45*(7), 1224-1230.

535 Ng, S. C., Hart, A. L., Kamm, M. A., Stagg, A. J., & Knight, S. C. (2009). Mechanisms
536 of action of probiotics: recent advances. *Inflammatory Bowel Diseases, 15*(2),
537 300-310.
538 Nguyen, T. D., Kang, J. H., & Lee, M. S. (2007). Characterization of *Lactobacillus*
539 *plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering
540 effects. *International Journal of Food Microbiology, 113*(3), 358-361.
541 Oliveira, M., Abadias, M., Colás-Medà, P., Usall, J., & Viñas, I. (2015). Biopreservative
542 methods to control the growth of foodborne pathogens on fresh-cut lettuce.
543 *International Journal of Food Microbiology, 214*, 4-11.
544 Pandey, A., Soccol, C. R., & Mitchell, D. (2000). New developments in solid state
545 fermentation: I-Bioprocesses and products. *Process Biochemistry, 25*, 1153-
546 1169.
547 Peng, M., Reichmann, G., & Biswas, D. (2015). *Lactobacillus casei* and its byproducts
548 alter the virulence factors of foodborne bacterial pathogens. *Journal of*
549 Qin, G., Tao, S., Cao, Y., Wu, J., Zhang, H., Huang, W., & Zhang, S. (2012).
550 Evaluation of the volatile profile of 33 *Pyrus ussuriensis* cultivars by HS-SPME
551 with GC–MS. *Food Chemistry, 134*(4), 2367-2382.
552 Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer
553 perception and choice of minimally processed vegetables and packaged fruits.
554 *Food Quality and Preference, 15*(3), 259-270.
555 Rizzolo, A., Cambiaghi, P., Grassi, M., & Zerbini, P. E. (2005). Influence of 1-
556 methylcyclopropene and storage atmosphere on changes in volatile compounds
557 and fruit quality of conference pears. *Journal of Agricultural and Food*
558 *Chemistry, 53*(25), 9781-9789.
559 Rößle, C., Auty, M. A. E., Brunton, N., Gormley, R. T., & Butler, F. (2010a). Evaluation
560 of fresh-cut apple slices enriched with probiotic bacteria. *Innovative Food*
561 *Science & Emerging Technologies, 11*(1), 203-209.

562 Rößle, C., Brunton, N., Gormley, R. T., Ross, P. R., & Butler, F. (2010b). Development
563 of potentially synbiotic fresh-cut apple slices. *Journal of Functional Foods, 2*(4),
564 245-254.
565 Russo, P., de Chiara, M. L. V., Vernile, A., Amodio, M. L., Arena, M. P., Capozzi, V.,
566 Massa, S., & Spano, G. (2014). Fresh-cut pineapple as a new carrier of
567 probiotic lactic acid bacteria. *Biomed Research International, 2014*, 1-9.
568 Russo, P., Pena, N., de Chiara, M. L. V., Amodio, M. L., Colelli, G., & Spano, G.
569 (2015). Probiotic lactic acid bacteria for the production of multifunctional fresh-
570 cut cantaloupe. *Food Research International, 77*, 762-772.
571 Salmeron, I., Loeza-Serrano, S., Perez-Vega, S., & Pandiella, S. S. (2015). Headspace
572 gas chromatography (HS-GC) analysis of imperative flavor compounds in
573 *Lactobacilli*-fermented barley and malt substrates. *Food Science and*
574 *Biotechnology, 24*(4), 1363-1371.
575 SAS Institute, I. (2004). SAS/STAT© 9.1 User's Guide. SAS Institute, Inc., Cary, NC.
576 Schieberle, P., Ofner, S., & Grosch, W. (1990). Evaluation of potent odorants in
577 cucumbers (*Cucumis sativus*) and muskmelons (*Cucumis melo*) by aroma
578 extract dilution analysis. *Journal of Food Science 5*, 193-195.
579 Siroli, L., Patrignani, F., Serrazanetti, D. I., Tabanelli, G., Montanari, C., Gardini, F., &
580 Lanciotti, R., (2015). Lactic acid bacteria and natural antimicrobials to improve
581 the safety and shelf-life of minimally processed sliced apples and lamb's lettuce.
582 *Food Microbiology, 47*, 74-84.
583 Siroli, L., Patrignani, F., Serrazanetti, D. I., Tappi, S., Rocculi, P., Gardini, F., &
584 Lanciotti, R., (2015). Natural antimicrobials to prolong the shelf-life of minimally
585 processed lamb's lettuce. *Postharvest Biology and Technology, 103*, 35-44.
586 Soliva-Fortuny, R. C., Alos-Saiz, N., Espachs-Barroso, A., & Martin-Belloso, O. (2004).
587 Influence of maturity at processing on quality attributes of fresh-cut conference
588 pears. *Journal of Food Science, 69*(7), 290-294.

589 Sreekumar, R., Al-Attabi, Z., Deeth, H. C., & Turner, M. S. (2009). Volatile sulfur
590 compounds produced by probiotic bacteria in the presence of cysteine or
591 methionine. *Letters in Applied Microbiology, 48*(6), 777-782.
592 Ukuku, D. O., Geveke, D. J., Chau, L., & Niemira, B. A. (2016). Microbial safety and
593 overall quality of cantaloupe fresh-cut pieces prepared from whole fruit after wet
594 steam treatment. *International Journal of Food Microbiology*, *231*, 86–92.
595 Wyllie, S. G., Leach, D. N., Wang, Y. M., & Shewfelt, R. L. (1995). Key aroma
596 compounds in melons: Their development and cultivar dependence. In R. L.
597 Rouseff & M. M. Leahy (Eds.), *Fruit Flavors: Biogenesis, Characterization, and*
598 *Authentication* (pp. 248-257). Washington, DC: American Chemical Society.
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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.

Figure

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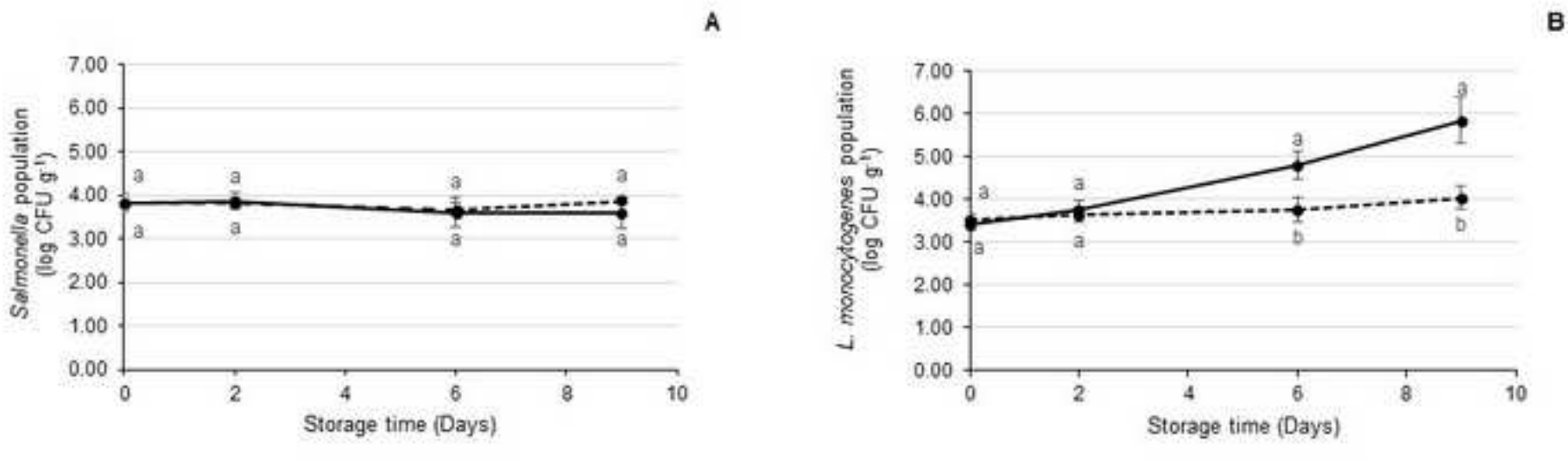

Figure 1

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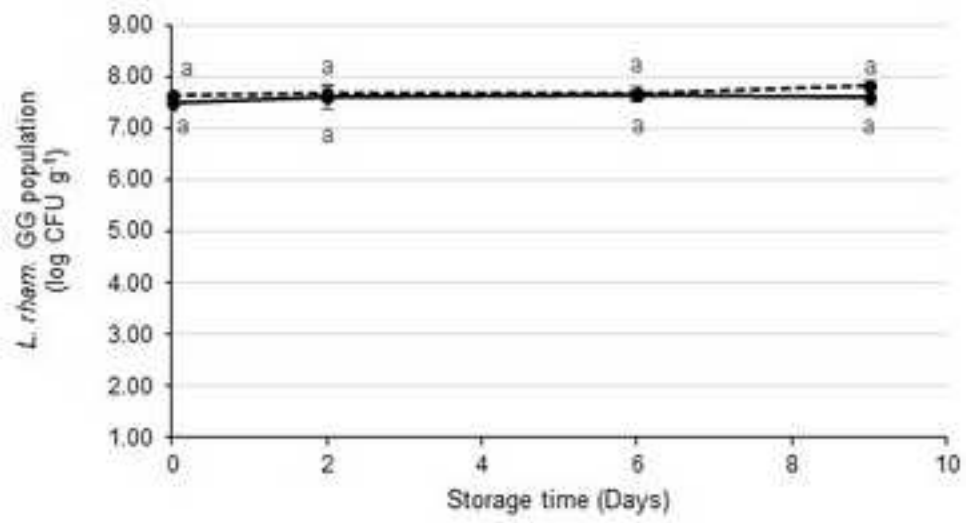

Figure 2

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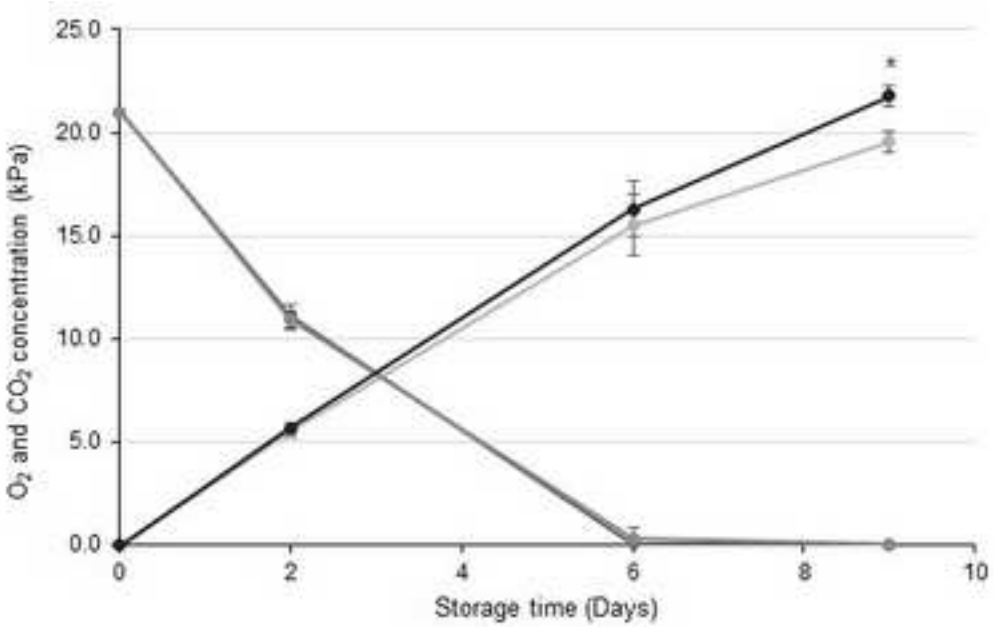

Figure 3

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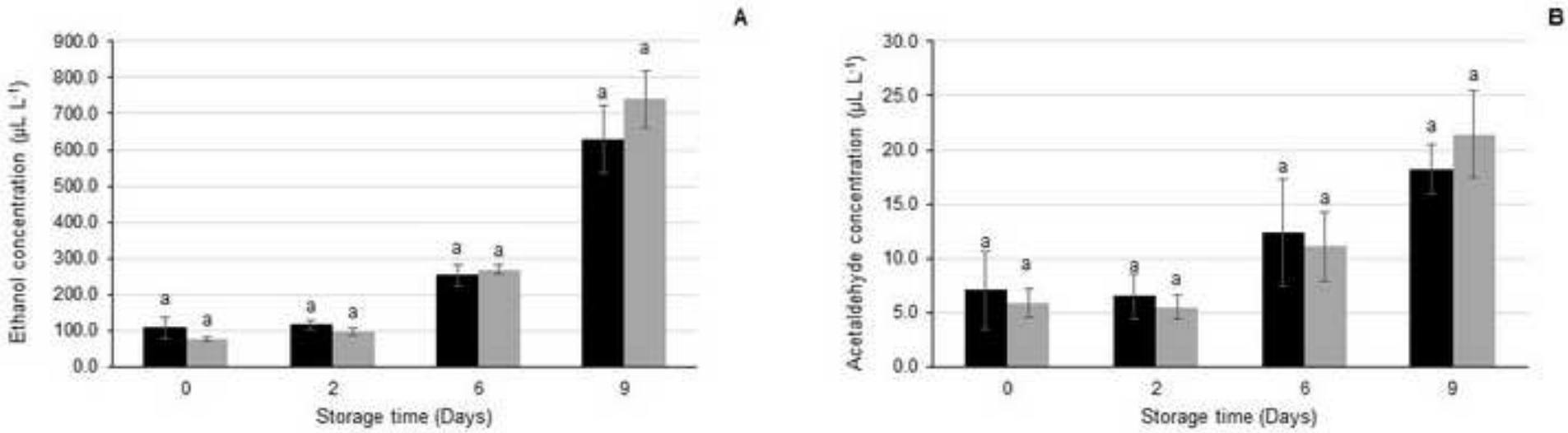

Figure 4

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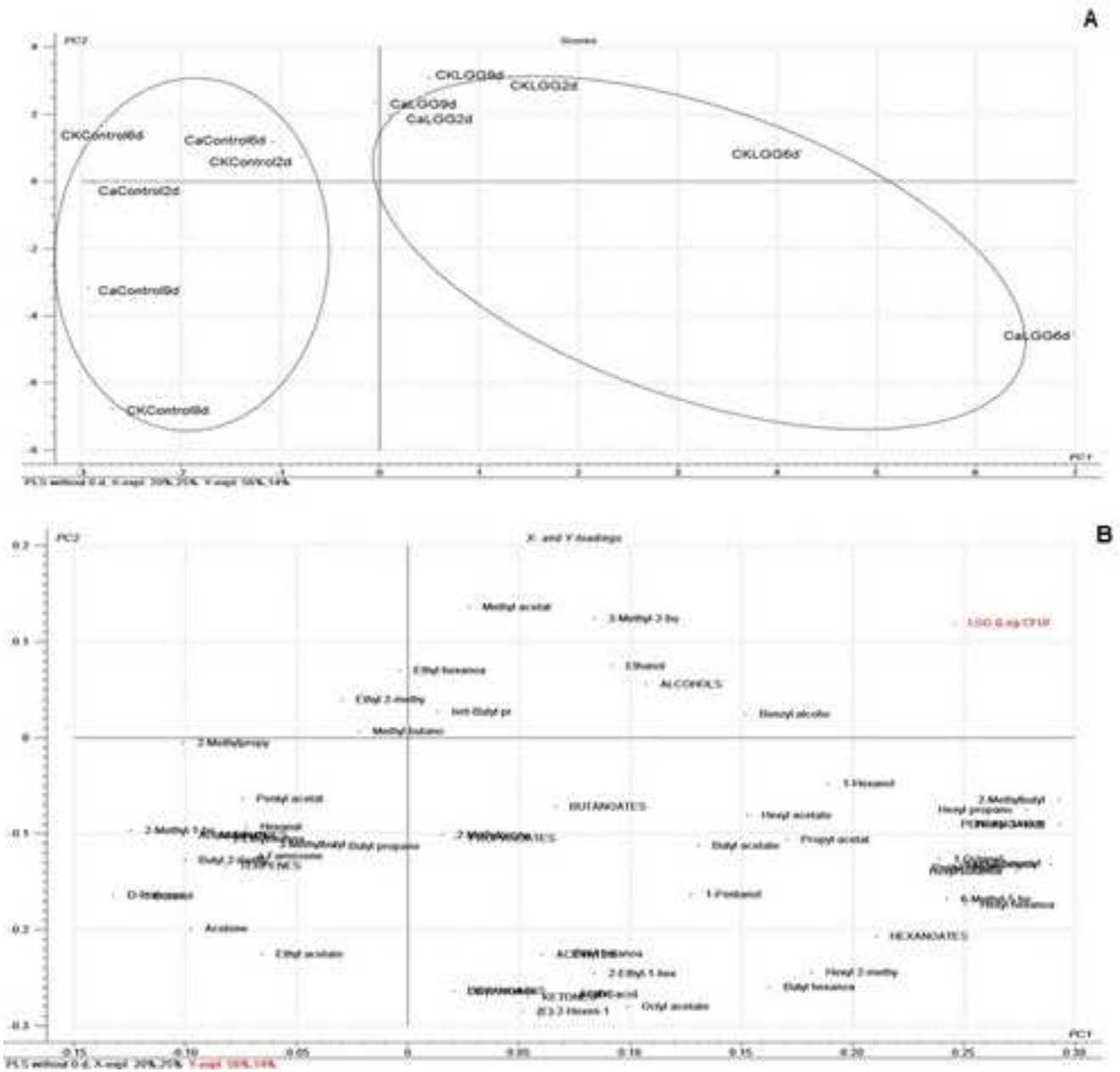

Figure 5

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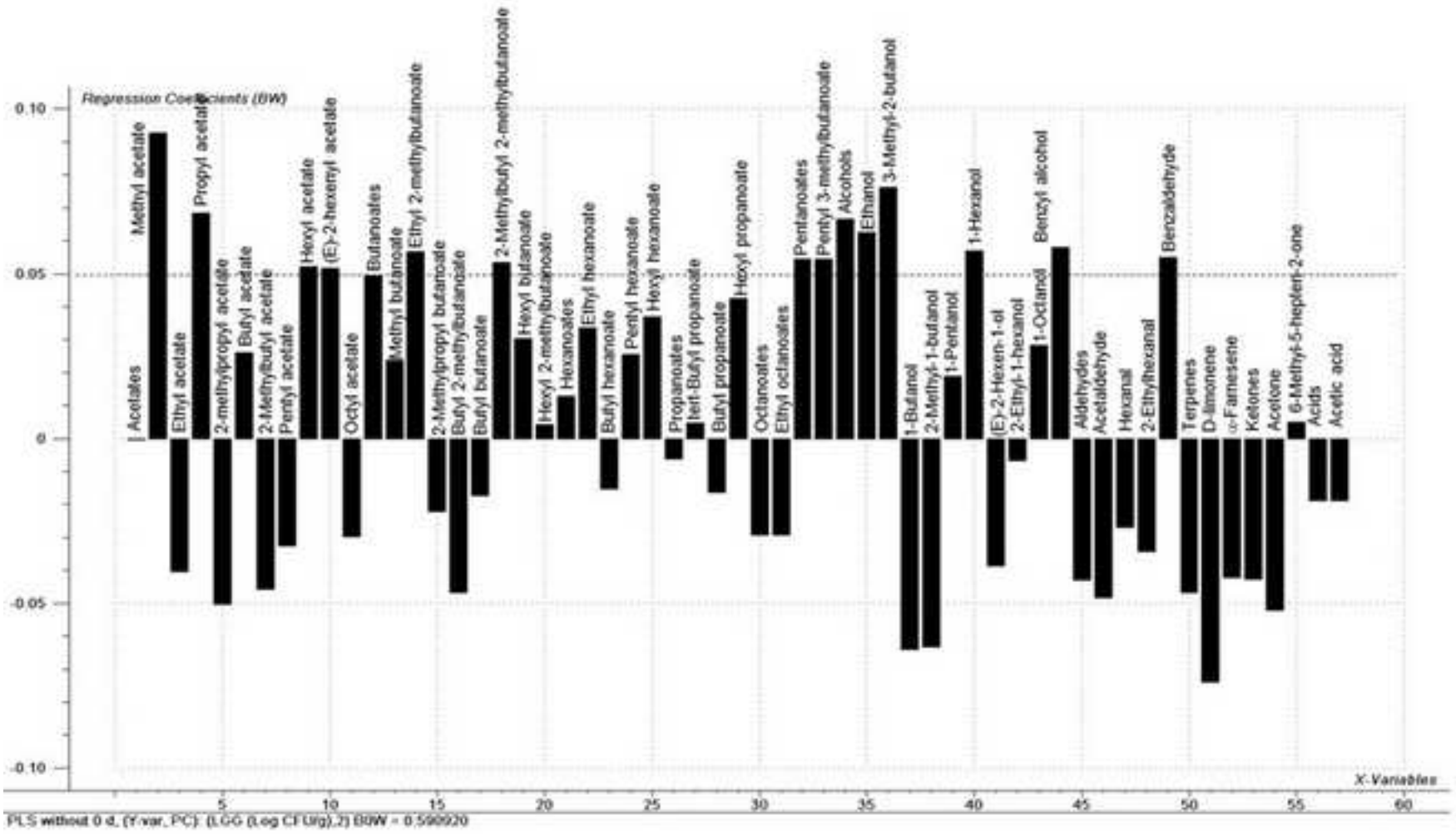

Figure 6

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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