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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.

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, Colás-Medà, 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

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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. (2015a,b) 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, 2010).

Salmeron, Loeza-Serrano, Perez-Vega, and 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), Gaminara (ATCC BAA-711) and Enteritidis (CECT 4300). Each *Salmonella* strain was grown individually in tryptone soy broth (TSB, Oxoid) medium for 20–24 h at 37 ± 1 °C.

A cocktail of five *Listeria monocytogenes* serovars was used: serovar 1a (CECT 4031), serovar 3a (CECT 933), serovar 4d (CECT 940), serovar 4b (CECT 4032) and serovar 1/2a, which was previously isolated in our laboratory from a fresh-cut lettuce sample (Abadias, Usall, Anguera, Solsona, & Viñas, 2008). *L. monocytogenes* strains were grown individually in TSB supplemented with 6 g L^{-1} of yeast extract (tryptone yeast extract soy broth, TSBYE) for 20–24 h at 37 ± 1 °C. Bacterial cells were harvested by centrifugation at 9800 × g, 10 min at 10 °C. The broth was decanted and the cells were resuspended in saline solution (SS; 8.5 g L^{-1} NaCl). Equal volumes of the five *Salmonella* concentrated suspensions were mixed to produce a single suspension, as well as the five *L. monocytogenes* concentrated suspensions.

The antagonist used in this study was the probiotic strain *Lactobacillus rhamnosus* GG (ATCC 53103) (*L. rhamnosus* GG) (from Ashtown Food research Centre, Teagasc, Ashtown, Dublin, Ireland). The antagonist was grown in de Man, Rogosa and Sharpe (MRS, Biokar Diagnostics, Beauvais, France) broth for 20–24 h at 37 ± 1 °C. Bacterial cells were harvested by centrifugation at 9800 × g, 10 min at 10 °C. The broth was decanted and the cells were resuspended in sterile distilled water.

For the inoculum preparation, an aliquot of each of the bacterial concentrated suspensions was added to deionised water to obtain approximately 10^5 CFU mL^{-1} in the case of *Salmonella* and *L. monocytogenes* and 10^8 CFU mL^{-1} for *L. rhamnosus* GG. Inoculum concentration was checked by plating appropriate dilutions onto XLD (Xylose-Lysine-Desoxycholate Agar, Oxoid) for *Salmonella*, onto Palcam agar (Palcam Agar Base with selective supplement, Biokar Diagnostics, Beauvais, France) for *L. monocytogenes* and onto MRS agar for *L. rhamnosus* GG. The plates were incubated at 37 ± 1 °C for 24 and 48 h for *Salmonella* and L. *monocytogenes*, respectively, and at 37 ± 1 °C for 48 h for the probiotic strain.

2.3. Inoculation of fruit and packaging

Prior to the experimental study, pears of both lots were washed in running tap water and surface disinfected with ethanol at 70%. They were peeled and cut into 10 wedges using a handheld apple slicer/corer. An antioxidant solution containing 20 g L^{-1} ascorbic acid, 20 g L^{-1} sodium citrate and 10 g L^{-1} $CaCl_2$ was used to prevent fresh-cut pear browning. Previous studies (data not shown) demonstrated that this antioxidant solution has no effect on bacteria viability. Pear wedges were dipped (1:2 w/v) for 2 min at 150 rpm according to the following treatments: (a) control (untreated): antioxidant solution (b) Sal + Lm: antioxidant solution inoculated with *Salmonella* and *L. monocytogenes* at 10^5 CFU mL^{-1} each, (c) *L. rhamnosus* GG: antioxidant solution inoculated with *L. rhamnosus* GG at 10^8 CFU mL^{-1} each or (d) Sal + Lm + *L. rhamnosus* GG: antioxidant solution inoculated with *Salmonella* and *L. monocytogenes* (10^5 CFU mL^{-1}) and *L. rhamnosus* GG (10^8 CFU mL^{-1}). Afterwards, they were allowed to dry in a laminar flow biosafety cabinet.

Pear wedges were packaged (110 ± 5 g) in passive atmosphere by placing them in 375-mL polypropylene trays and sealing with a non-peelable polypropylene plastic film (PP-110, ILPRA, Italy) of 64 μm in thickness with an O_2 permeability of 110 cm^3 m^{-2} day^{-1} atm^{-1} at 23 °C and a water steam permeability of 10 g m^{-2}