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Antagonistic effect of probiotic bacteria against foodborne pathogens on fresh-cut pear

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ABSTRACT

The use of probiotics as biopreservation agents of foodborne pathogens in food is becoming increasingly known. The aim of this work was to investigate the effectiveness of *Lactobacillus rhamnosus* GG (*L. rham.* GG) and *Lactobacillus acidophilus* LA-5 (*L. acidophilus* LA-5) against *Salmonella* and *Listeria monocytogenes* in minimally processed pears during storage at 5, 10 and 20 °C at conditions simulating commercial application. Pear wedges were artificially inoculated with a suspension containing *Salmonella*, *L. monocytogenes* and/or the probiotic strains *L. rham.* GG or *L. acidophilus* LA-5, packaged and stored at 5, 10 and 20 °C. Microorganisms were periodically enumerated. *L. acidophilus* LA-5 did not shown any effect against pathogens. *Salmonella* was affected by co-inoculation with *L. rham.* GG at 10 and 20 °C, which reduced the population approximately 2-log units. Moreover, *L. monocytogenes* population was reduced approximately 3-log units at each temperature in presence of *L. rham.* GG. Probiotic populations were maintained throughout the experiment around 10^7–10^8 CFU g^{-1}, which is in the range known to develop its probiotic role (10^6–10^9 CFU g^{-1}). Our results demonstrated that *L. rham.* GG is able to control *Salmonella* and *L. monocytogenes* growth on fresh-cut pear.

1. Introduction

Nowadays, the consumption of minimally processed (MP) fruits and vegetables is growing more and more up to the point that MP fruits represent one of the most rapidly expanding segments of the lightly treated refrigerated food market owing to their increased functionality (Del Nobile, Conte, Scrocco, & Brescia, 2009). MP fruits are fresh, raw fruits processed in order to supply a ready-to-eat food product. Their processing generally consist in peeling, cutting, slicing, shredding, trimming, washing (sanitation) step and packaging and storage at refrigeration conditions. Fresh-cut fruit must resemble the original, whole product as closely as possible in order to be commercially successful (Arias, Gonzalez, Lopez-Buesa, & Oria, 2008). Fruits have been considered as microbiologically safe due to their low pH. However, during their processing the risk of contamination increases and the release of nutrients due to cutting could stimulate microbial growth of epiphytic and spoilage microorganisms (Abadias, Alegre, Usall, Torres, & Viñas, 2011; Lanciotti et al., 2003). Pathogens could be introduced at any point of the production chain of fresh-cut fruit and they might be present when the produce is consumed. So, fresh-cut fruits can be a vehicle for the transmission of foodborne pathogens. In the last years, for example, *L. monocytogenes* outbreaks have been related to cantaloupe (CDC, 2016). Mango, papaya and cantaloupe were responsible for foodborne outbreaks due to *Salmonella* (CDC, 2016).

It is known that an efficient temperature control during manufacture, distribution and retailing in combination with modified atmosphere packaging (MAP) is required for maintaining the microbiological quality and the safety of these products (Abadias et al., 2014; Siroli et al., 2014), as well as the use of disinfectants to reduce bacterial populations (Abadias et al., 2011). However, the consumers concern about chemical additives gives cause for studying alternative methods to maintain safety and extend shelf-life of MP fruits. As alternatives to chemical products, there is literature in which natural products from plants and fruits are used as antimicrobials (Bubonja-Sonje, Giacometti, & Abram, 2011; Lacombe, McGivney, Tadepalli, Sun, & Wu, 2013; Lacombe, Wu, Tyler, & Edwards, 2010; Lacombe, Wu, White, Tadepalli, & Andre, 2012; Yang, Hewes, Salaheen, Federman, & Biswas, 2014). Authors such as Abadias et al. (2014), Alegre, Viñas, Usall, Anguera, and Abadias (2011), Leverentz et al. (2006) and Siroli et al. (2014)

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reported that some microorganisms could be used as biopreservation agents, controlling pathogens growth during shelf life.

On the other hand, the trend to consume more and more functional foods is increasing, in particular food enriched with probiotic microorganisms. A probiotic has been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Probiotic microorganisms are, mostly, *Lactobacillus* and *Bifidobacterium* which are types of lactic acid bacteria (LAB) (Sheehan, Ross, & Fitzgerald, 2007). *Lactobacillus rhamnosus* GG (*L. rham.* GG) is one of the most widely studied probiotics to prevent inflammatory bowel diseases (Dhanani, Gaudana, & Bagchi, 2011), reduce respiratory tract infections (Smith, Rigassio-Radler, Denmark, Haley, & Touger-Decker, 2013), enhance immune response (Szajewska, Kotowska, Mrukowicz, Armanska, & Mikolajczyk, 2001), and prevent skin diseases (Boyle et al., 2010). Usually they are added to dairy milk, but the interest in beverages and non-dairy products enriched with probiotic microorganisms is growing because they are an alternative option for people who are allergic to milk proteins, lactose intolerant, hypercholesterolemic or vegetarian (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Ranadheera, Baines, & Adams, 2010). Probiotic microorganisms and LAB have been described to be able to reduce foodborne pathogens in apples (Alegre et al., 2011; Siroli et al., 2015; Trias, Badosa, Montesinos, & Baneras, 2008a; Trias, Baneras, Badosa, & Montesinos, 2008b), pineapple (Russo et al., 2014) and cantaloupe (Russo et al., 2015), hence they can be used as biopreservation agents because LAB are considered as GRAS (Generally Recognized As Safe) (Siroli et al., 2015). Mechanisms for the inhibition of pathogens described are the production of inhibitory or antimicrobial substances, their acting as competitive antagonists such as competition for adhesion sites and nutrients and the stimulation of the immune system (Lourens-Hattingh & Viljoen, 2001).

'Conference' pear is the most produced variety in Spain. Pears are rich in micronutrients, minerals and antioxidants as well as have low protein and lipids contents but rich in sugar (Colás-Medà, Abadias, Alegre, Usall, & Viñas, 2015). The variety 'Conference' is the most suitable to obtain a MP product due to its low susceptibility to browning and its high sensorial acceptance (Arias et al., 2008; Soliva-Fortuny, Biosca-Biosca, Grigelmo-Miguel, & Martin-Belloso, 2002; Soliva-Fortuny, Grigelmo-Miguel, Hernando, Lluch, & Martin-Belloso, 2002).

The aim of this work was to test the effectiveness of probiotic bacteria, *L. rham.* GG and *L. acidophilus* LA-5 against *Salmonella* and *L. monocytogenes* at conditions simulating commercial application throughout storage at different temperatures.

2. Materials and methods

2.1. Fruit

'Conference' pears (*P. communis* L. cv. Conference) were obtained from local packinghouses in Lleida (Catalonia). The fruits were stored at 0 °C until use. The pears were ripened by incubation at 20 °C until the optimum ripeness stage for processing (44 ± 3.2 N) according to Soliva-Fortuny, Alos-Saiz, Espachs-Barroso, and Martin-Belloso (2004). Prior to experimental studies, pears were washed in running tap water and surface disinfected with ethanol 70% and let to dry at room temperature. They were cut in 10 skin-off wedges using an apple handheld slicer/corer.

2.2. Bacterial strains

The bacterial strains used in this work were the serovars of *Salmonella enterica* subsp. *enterica*: Agona (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC BAA-710), Gaminara (ATCC BAA-711) and Enteritidis (CECT-4300) and *Listeria monocytogenes* 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). The probiotic strains used in this study were *Lactobacillus rhamnosus* GG (ATCC 53103) (*L. rham.* GG) (from Ashtown Food research Centre; Teagasc; Ashtown, Dublin, Ireland) and *Lactobacillus acidophilus* LA-5 (*L. acidophilus* LA-5) (Chr. Hansen Hørsholm, Denmark). They were grown in de Man, Rogosa and Sharpe (MRS, Biokar Diagnostics, Beauvais, France) broth for 20–24 h at 37 ± 1 °C. *Salmonella* strains were grown individually in tryptone soy broth (TSB, Oxoid) medium for 20–24 h at 37 ± 1 °C. *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 sterile distilled water (*L. rham.* GG and *L. acidophilus* LA-5) or saline solution (SS; 8.5 g L^{-1} NaCl, *Salmonella* and *L. monocytogenes*). Equal volumes of the five *Salmonella* concentrated suspensions were mixed to produce a single suspension, as well as the five *L. monocytogenes* concentrated suspensions.

For the inoculum preparation, a volume of each of the bacterial concentrated suspensions was added to deionized 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. rham.* 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. rham.* GG and *L. acidophilus* LA-5. The plates were incubated at 37 ± 1 °C for 24 h for *Salmonella*, 48 h for *L. monocytogenes* and probiotic strains.

2.3. Fresh-cut pear treatment

To prevent fresh-cut pear browning, 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. Pear wedges were dipped (1:2 w/v) for 2 min at 150 rpm according to the following treatments: (a) Sal + Lm: antioxidant solution inoculated with *Salmonella* and *L. monocytogenes* at 10^5 CFU mL^{-1} each, (b) *L. rham.* GG or LA-5: antioxidant solution inoculated with *L. rham.* GG or *L. acidophilus* LA-5 at 10^8 CFU mL^{-1} each or (c) Sal + Lm + *L. rham.* GG or LA-5: antioxidant solution inoculated with *Salmonella* and *L. monocytogenes* (10^5 CFU mL^{-1}) and *L. rham.* GG or *L. acidophilus* LA-5 (10^8 CFU mL^{-1}). Afterwards they were allowed to dry in a laminar flow biosafety cabinet.

In previous studies (data not shown) we observed that the antioxidant solution tested did not affect *Salmonella*, *L. monocytogenes*, *L. rham.* GG and *L. acidophilus* LA-5 viability.

Approximately 8 pear wedges (110 ± 5 g) were packaged 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} day^{-1} at 23 °C and 90% relative humidity (ILPRA). Pear trays were stored at 20, 10 and 5 °C.

2.4. Enumeration of bacterial concentration

Populations of *Salmonella*, *L. monocytogenes*, *L. rham.* GG and *L. acidophilus* LA-5 were determined in three sample trays for each