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Probiotic lactic acid bacteria for the production of multifunctional fresh-cut cantaloupe

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ABSTRACT

Minimally processed fruits are an ideal alternative to dairy products to deliver probiotic microorganisms. At the same time, several innovative employments of lactic acid bacteria (LAB) have been proposed in the food industry, including bio-fortification with nutritional compounds and bio-protection against foodborne pathogenic bacteria. In this study, probiotic riboflavin over-producing *Lactobacillus plantarum* B2 and *Lactobacillus fermentum* PBCC11.5 were inoculated on fresh-cut cantaloupe by immersion in a dipping solution. The viability of probiotic microorganisms and the main physico-chemical parameters of melon pieces, including the riboflavin content, were monitored for 11 days of storage under refrigerated conditions. Finally, both probiotics were tested for their antagonistic effect against different concentrations of an isolate of *Listeria monocytogenes* from fruit origin. Overall, high viability of both probiotics species was found at the end of the shelf life. The main technological and nutritional parameters of the fruits were unaffected by probiotic-enrichment, except some sensorial attributes when melons were inoculated with *L. plantarum* B2. The riboflavin content increased about two-fold in probiotic cantaloupe. Moreover, *L. plantarum* B2 and *L. fermentum* PBCC11.5 showed a good ability to reduce the level of *L. monocytogenes* on artificially contaminated melons. In conclusion, the results of this work encourage further implementation of new foods with multifunctional properties.

1. Introduction

Fresh-cut vegetables and fruits are an expanding sector of the food industry. Consumers generally have high expectation for the quality of these minimally processed foods, in terms of freshness, nutritional value, organoleptic acceptance, healthful, and convenience. Therefore, in the last years, a number of innovative strategies, including technological and microbial approaches, have been suggested to maximize their quality and shelf life (Dhall, 2013; Francis et al., 2012).

Probiotic fortification is a well-established approach to produce foods with functional properties. In this background, probiotic vegetables and fruits are considered a promising alternative to probiotic dairy products, since these food formulations can better meet the wants of particular categories of consumers, as vegetarians and vegans, lactose intolerants, individuals with low-cholesterol intake need, or allergic to animal proteins (Gupta & Abu-Ghannam, 2012). In the last years, several products of vegetable origin have been suggested for the consumption of probiotic bacteria including purées, table olives, kimchi, and fermented juices (Di Cagno, Coda, Angelis, & Gobbetti, 2013; Martins et al., 2013; Prado, Parada, Pandey, & Soccol, 2008). However, the use of minimally processed fruits as carriers of beneficial microbes was restricted to a limited range of products including probiotic-enriched fresh-cut papaya, apple, and pineapple (Alegre, Viñas, Usall, Anguera, & Abadias, 2011; Rößle, Auty, Brunton, Gormley, & Butler, 2010; Russo, de Chiara, et al., 2014; Tapia et al., 2007). In contrast, several studies aimed at determining the probiotic potential of autochthonous lactic acid bacteria (LAB) isolated from fruit and vegetables throughout the world (Lee et al., 2011; Tamang, Tamang, Schillinger, Guigas, & Holzapfel, 2009; Vitali et al., 2012). This effort should identify the availability of excellent probiotic candidates well adapted to the typical stressors of minimally processed vegetables (Capozzi, Fiocco, Amodio, Gallone, & Spano, 2009). Moreover, the recent advances in comparative genomic of LAB provided valuable insights to select new strains with interesting properties for food and health applications (Douillard & de Vos, 2014; Sánchez, Ruiz, Gueimonde, & Margolles, 2013). Among a number of beneficial effects, vitamin-producing starter cultures and probiotic strains could be a cost-effective alternative to current vitamin fortification strategies and be suitable in the elaboration of novel vitamin-enriched products (Capozzi, Russo, Dueñas, López, & Spano, 2012; Leblanc et al., 2011). Moreover, LAB have the status of qualified presumption of safety (QPS) (EFSA, 2013), thus requiring a more concise assessment before their introduction into the food chain.

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In addition, some LAB strains have been recently proposed as hopeful bioprotective cultures in order to improve the microbial safety of fresh-cut products (Olaimat & Holley, 2012; Ramos, Miller, Brandão, Teixeira, & Silva, 2013). From a safety point of view, minimally processed fruits and vegetables are a potential vehicle of transmission of foodborne pathogens, mainly including *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. In particular, melons have been frequently implicated in produce-associated outbreaks (Walsh, Bennett, Mahovic, & Gould, 2014). In recent years, cantaloupes contaminated with *L. monocytogenes* were the causative agents of the deadliest foodborne outbreak in the United States since the 1920s (CDC, 2011), underscoring the importance to adopt strategy to contrast contamination and growth of this pathogen. Among non-thermal processes, biopreservation is a promising eco-friendly approach based on complex competition phenomena among deliberately added beneficial microbes and foodborne pathogens (Abadias et al., 2014; Alegre et al., 2011, 2013; Russo, de Chiara, et al., 2014; Vignolo, Saavedra, Sesma, & Raya, 2012).

In the present work, two riboflavin overproducing LAB strains, previously characterized for their probiotic potential, were investigated for the production of a functional fresh-cut cantaloupe. Viability of probiotic bacteria and the main physico-chemicals parameters of melon fruit pieces, including the vitamin B2 content, were monitored throughout the shelf life of the product. Finally, the antagonistic effect of the probiotic microorganisms against a strain of *L. monocytogenes* from vegetable origin was also tested in order to enhance the safety of minimally processed cantaloupe.

2. Material and methods

2.1. Bacterial strains and growth conditions

Lactobacillus plantarum B2 and *Lactobacillus fermentum* PBCC11.5 (Arena et al., 2014) were routinely grown on MRS broth (Oxoid, Hampshire, UK) at 37 °C. These strains were previously deposited at the Spanish Type Culture Collection (CECT, Paterna, Spain) under the code number CECT 8328 and CECT 8448, respectively. *L. monocytogenes* A.9.4 (serotype 4b) from strawberries origin was kindly provided by the University of Athens and grown on brain heart infusion (BHI) at 37 °C.

2.2. Preparation of the probiotic solution

A probiotic solution containing *L. plantarum* B2 or *L. fermentum* PBCC11.5 was obtained as previously reported (Russo, de Chiara, et al., 2014). Briefly, probiotic microorganisms were grown in 2 L of MRS until the late exponential phase (5×10^9 CFU mL^{-1}). Cells were washed twice with citric acid-sodium citrate buffer (pH 3.8) (Sigma-Aldrich, St. Louis, MO, USA), and resuspended in 2 L of the same buffer. Inoculum concentration was checked by plating appropriate dilutions onto MRS agar plates.

2.3. Inoculation of cantaloupe with probiotic bacteria

Cantaloupe melons (*Cucumis melo* var. *cantaloupensis*) used for this study were purchased at local market (Foggia, Italy). Fruits harvested at commercial maturity were sorted to eliminate damaged or defective fruit, cleaned in chlorinated water (1 μL L^{-1} sodium hypochlorite), and washed in tap water and dried. The skin was manually removed using a ceramic knife, the blossom and stem ends were discarded, placental tissue and seeds were removed and the pulp cut into 1-cm-thick wedges. From each wedge 6 pieces were obtained (approximately 3.5 × 2 cm). Then, 45 pieces for each treatment were dipped (2 min with shaking) on 650 mL of buffer solution containing *L. plantarum* or *L. fermentum*, respectively. Control samples were dipped on citric acid-sodium citrate buffer without probiotics. Finally, fresh-cut melons were air-dried, and packed in polypropylene plastic film bags (10 × 10 cm, OTR of 1100 $cm^3 \cdot m^2\ 24\ h^{-1}\ bar^{-1}$), each containing 15 pieces. Bags were thermally sealed in passive-modified atmosphere packaging and stored at 4 °C. At 0, 4, 8 and 11 days of storage, the main physico-chemical attributes of cantaloupe melon pieces and the probiotic viability were monitored on both inoculated and non inoculated samples. All experiments were carried out in triplicate.

2.4. Enumeration of probiotic

The viability of the probiotic was monitored at 0, 4, 8, and 11 days of storage at 4 °C. Two pieces of cantaloupe samples were weighted, diluted (1:10) in saline solution (NaCl 8.6 g L^{-1}), and homogenized in a blender (Bag Mixer, Interscience, Saint-Nom-la-Bretèche, France) for 2 min. To enumerate probiotic strains, tenfold serial dilution was plated onto MRS agar and incubated at 37 °C for 48 h. The concentration of mesophilic microorganism was determined on PCA (Oxoid), after incubation at 25 °C for 48 h. The concentration of yeasts and molds was determined on PDA (Oxoid) added with chloramphenicol (100 mg L^{-1}), after incubation at 25 °C for 48 h.

2.5. Color analysis

Color was measured by elaborating the images acquired with a Spectral scanner (DV SRL, Italia). The external surface of eight melon pieces for each replicate was scanned. The central region was manually selected. On these regions, color in CIE L^*, a^*, and b^* scale was measured. From the primary L^*, a^*, and b^* values the following indexes were calculated:

- Hue angle ($h° = \arctan \frac{b^*}{a^*}$)
- Chroma $= \sqrt{a^{*2} + b^{*2}}$
- Global color variation $\Delta E = \sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2}$.

2.6. Gas composition

Oxygen and carbon dioxide percentage inside the bags was measured in 15 mL headspace gas sample using a handheld gas analyzer (CheckPoint, Dansensor A/S, Denmark) during the storage time.

2.7. Firmness

To assess firmness of fresh-cut melons, 10 pieces for each replicate were taken and cut into small cubes (10 mm side length). These cubes were compressed between two parallel plates using an Instron Universal Testing Machine (model 3340), with a crosshead speed of 30 mm ∗ min^{-1}. Firmness of the fruit samples was defined as the rupture load of the force/deformation curve and expressed in Newton (N).

2.8. Vitamin B_2

The riboflavin content of cantaloupe was analytically determined as previously described (Russo, Capozzi, et al., 2014). Briefly, 5–10 g of samples was added with 25 mL 0.1 M HCl and submitted to acid hydrolysis by autoclaving at 121 °C for 30 min. Then, pH was adjusted to 4.5 with 4 M sodium acetate and samples were submitted to enzymatic treatment by adding a 5-mL solution containing α-amylase (420 U), papain (12 U), acid phosphatase (22 U), and 0.1% of glutathione (all purchased from Sigma Aldrich). After 1 h of exposure at an ultrasonic bath, samples were diluted up to 50 mL with 0.01 M HCl, and submitted to HPLC quantification according to Jakobsen (2008).

2.9. Vitamin C

Vitamin C content was assessed homogenizing 5 g of melon tissues in an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 1 min