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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

with 5 mL of methanol/water (5:95), plus citric acid (21 g L^{-1}), EDTA (0.5 g L^{-1}), and NaF (0.168 g L^{-1}). The homogenate was filtered through cheesecloth and the pH adjusted to 2.2–2.4 by addition of 6 mol L^{-1} HCl. The homogenate was centrifuged at 10,000 rev^{-1} for 5 min and the supernatant was recovered, filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA) and then through a 0.2 μm cellulose acetate filter. L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA) contents were determined as described by Zapata and Dufour (1992) with some modifications (Gil, Ferreres, & Tomás-Barberán, 1999). The HPLC analysis was achieved after derivatization of DHA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of 20 μL were analyzed with an HPLC (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) equipped with a DAD detector and a binary pump. Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm; 5 μm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H2O (5:95 *v*/*v*) containing 5 mmol L^{-1} cetrimide and 50 mmol L^{-1} potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL min^{-1}. The detector wavelengths were 348 nm for DHA and 251 nm for AA. AA and DHAA contents were expressed as mg of L-ascorbic or L-dehydroascorbic acid per 100 g of fresh weight.

2.10. Total phenols and antioxidant capacity

To determine the total phenol content, 5 g of melon tissues was homogenized in an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA) for 1 min in methanol:water solution (80:20) 2 mmol L^{-1} in sodium fluoride for 1 min. The homogenate was then centrifuged at 5 °C and 9000 rpm for 10 min. Total phenols were determined according to the method of Singleton and Rossi (1965). Each extract (100 μL) was mixed with 1.58 mL water, 100 μL of Folin–Ciocalteu reagent and 300 μL of sodium carbonate solution (200 g L^{-1}). After the solution stood for 2 h, the absorbance was read at 725 nm against a blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). The content of total phenols was calculated on the basis of the calibration curve of gallic acid, and was expressed as milligrams of gallic acid per 100 g of fresh weight (mg GA 100 g^{-1}). Antioxidant assay was performed following the procedure described by Brand-Williams, Cuvelier, and Berset (1995) with minor modifications. The diluted sample, 50 μL, was pipetted into 0.950 mL of DPPH solution to initiate the reaction. The absorbance was read at 515 nm after overnight incubation. Trolox was used as a standard and the antioxidant activity was reported in mg of Trolox equivalents per 100 g of fresh weight (mg TE 100 g^{-1}).

2.11. Simultaneous analysis of organic acids and sugars

Organic acids and sugars were extracted homogenizing 5 g of fresh melon tissues with 10 mL of ultrapure water using IKA T18 Ultraturrax (Wilmington, USA) homogenizer at 14,000 rpm for 1 min. The homogenate was centrifuged at 9000 rpm for 10 min at 5 °C. The supernatant was filtered with a C_{18} Sep-Pak cartridge (Grace Pure ™, New York, USA) and then with a 0.2 μm filter (INCOFAR, Modena, Italy). All extracts were performed in triplicate samples. Organic acids and sugars were identified using the method as described by Mena et al. (2011). Samples were diluted with ultrapure water (1:1) and were injected (10 μL) into HPLC system (Agilent 1200 series) equipped with an UV detector, set at 210 nm, coupled with a refractive index detector. Peak separation was achieved on a Rezex ROA-Organic Acid H+(8%) column (300 × 7.80 mm) (Phenomenex, Torrance, USA), using a mobile phase of acidified water (phosphoric acid (0.1%)) with a flow rate of 0.5 mL/min and an oven temperature of 30 °C. The different organic acids and sugars were characterized and quantified by chromatographic comparison with analytical standards. Sugar and organic acid contents were expressed as mg per 100 g of fresh weight.

2.12. Total soluble solids, titratable acidity, and pH

Few drops of melon puree were used to measure the total soluble solids content (TSS) with a digital hand refractometer (Atago, Japan) whereas 5 g was used to measure the pH and the titratable acidity (TA), with an automatic titrator (T50 M Terminal, Mettler Toledo, Switzerland). TA was obtained measuring the volume of NaOH 0.1 N used to reach a final pH of 8.2, and results were expressed as per cent of citric acid referred to the juice.

2.13. Acetaldehyde and ethanol

For this analysis 10 g of homogenized sample was put into 22 mL glass test tube, sealed with rubber stopper and stored at −20 °C freezer until analysis (Mateos, Ke, Cantwell, & Kader, 1993). After 1 h incubation at 65 °C water bath, a 0.5 mL headspace gas sample was taken and injected into gas chromatograph (Shimadzu GC-14A; FID temp. was 150 °C, injector temp. was 130 °C, oven temp. was 80 °C. 5% CBWX 20 M on Carbograph 1AW20 80/120, 6' × 1/8″ × 0.085″ AT STEEL column (Alltech)). Ethanol and acetaldehyde were identified by co-chromatography with standards and quantified by a range of concentrations of ethanol and acetaldehyde in 5 mL of water. Acetaldehyde and ethanol concentrations were reported in nmol g^{-1}.

2.14. Sensorial quality

A panel of three people carried out the sensory evaluations of fresh-cut melon at the processing day, and at each sampling time. Before evaluations, panelists were trained in order to recognize and score the quality attributes of the melon pieces. Samples were kept at 5 °C until sensorial evaluation, and each panelist was presented with 3 pieces from each treatment in a lidded container to avoid loss of aroma. The samples were coded with a random 3-digit number in order to mask the treatment identity and to minimize subjectivity. The sensorial attributes judged during evaluation were: odor, off odor, overall appearance, color, firmness, herbaceous, sweetness, and off flavor. The visual quality analysis was performed to evaluate the overall changes in flesh of melon samples after cutting and during shelf-life. It was used as an hedonic scale, including 5 pictures, each one associated with a brief description that corresponded to a score from 1 to 5 where 1 = *really poor, Mushy appearance, severe tissue damages, possible bacterial and/or fungal spoilage.*; 2 = *evident water soaked tissues* (*limit of edibility*); 3 = *slightly pale flesh, noticeable water soaked areas, start of softening* (*limit of marketability*); 4 = *fresh appearance with minor symptoms of translucency on tissue edges, firm texture, and* 5 = *excellent, fresh appearance, bright color, firm texture*. Every attribute was scored on a 1 to 5 scale, where 1 = *absent*, 3 = *moderate*; 5 = *full characteristic or fresh.*

2.15. Statistical analysis

The effect on quality parameters of treatment was tested by performing a two-way ANOVA using StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA), for both treatment and storage time, and their interaction, on monitored parameters. Moreover mean values within each sampling day were subjected to one-way ANOVA for the treatment. Mean values were separated applying Tukey test with significant difference when $P \leq 0.05$.

2.16. Antagonistic assays

Antagonistic assays were carried out as previously reported (Russo, de Chiara, et al., 2014). Briefly, from cantaloupe wedges, cylinders of melon (1.5 cm × 1.5 cm) were obtained with a corel. Cultures of probiotic LAB and *L. monocytogenes* strains at late exponential phase (about 10^9 CFU mL^{-1}) were centrifuged (5000 rpm × 5 min), and resuspended in sterile saline solution. Then, fruit samples were artificially

contaminated with probiotic, pathogenic bacteria, or both by spreading appropriate concentration of microbial cultures on the cylinder surface. In particular, the antagonistic effect of Lp B2 and PBCC11.5 was evaluated by co-inoculating 5×10^8 CFU g^{-1} of probiotic and three different concentrations (namely, 5×10^8; 5×10^6; 5×10^4 CFU g^{-1}) of *L. monocytogenes* R.9.4.

Viability of probiotic LAB and *L. monocytogenes* strains was monitored at 0, 2, 5, 7, and 9 days of storage at 4 °C. For enumeration,

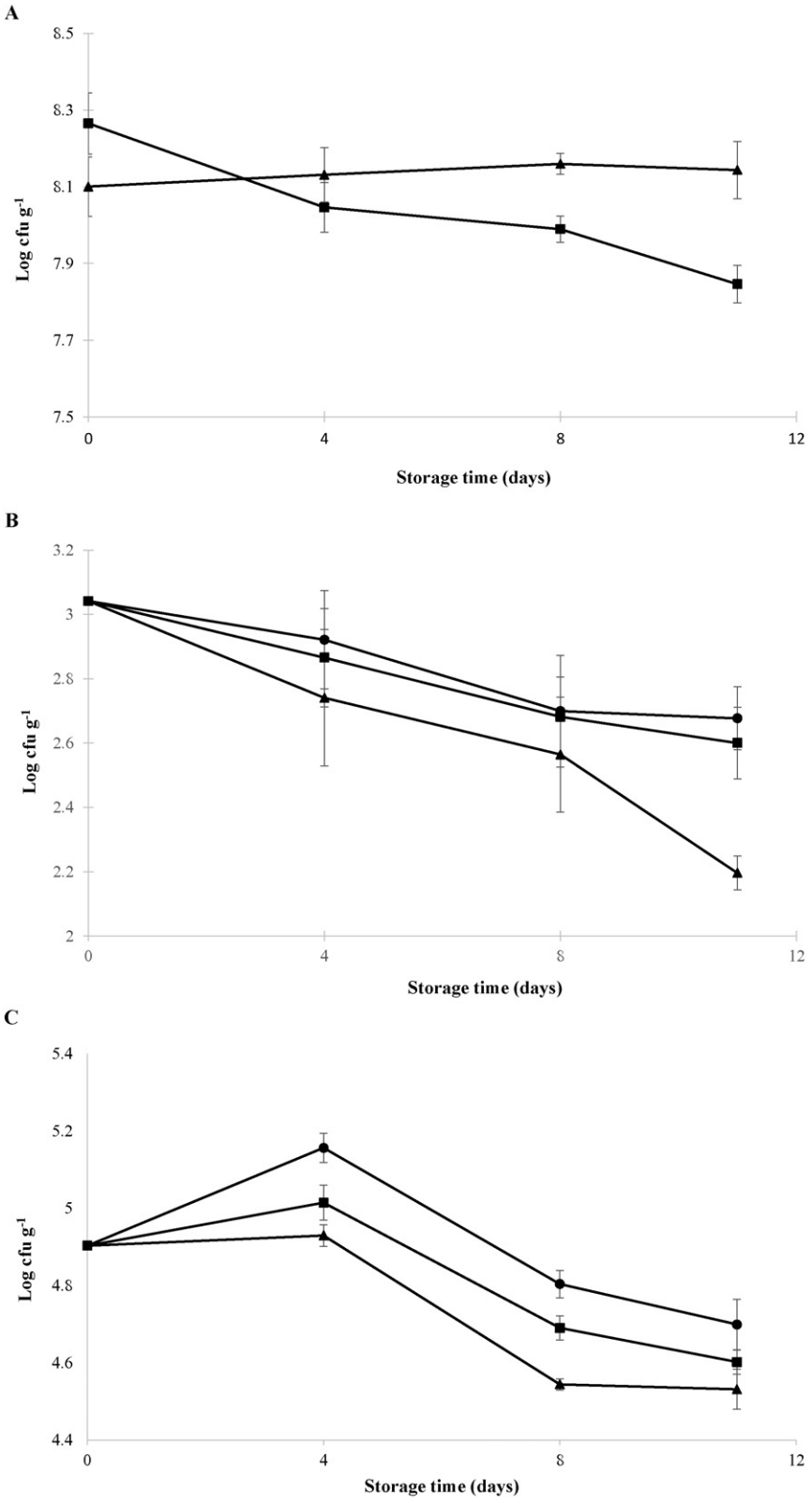

Fig. 1. The viability of probiotic (A), mesophilic (B) and yeasts and molds (C) was monitored after 0, 4, 8, and 11 days of storage at 4 °C in samples inoculated with *L. plantarum* B2 (triangle), *L. fermentum* PBCC11.5 (square), or not inoculated (circle).

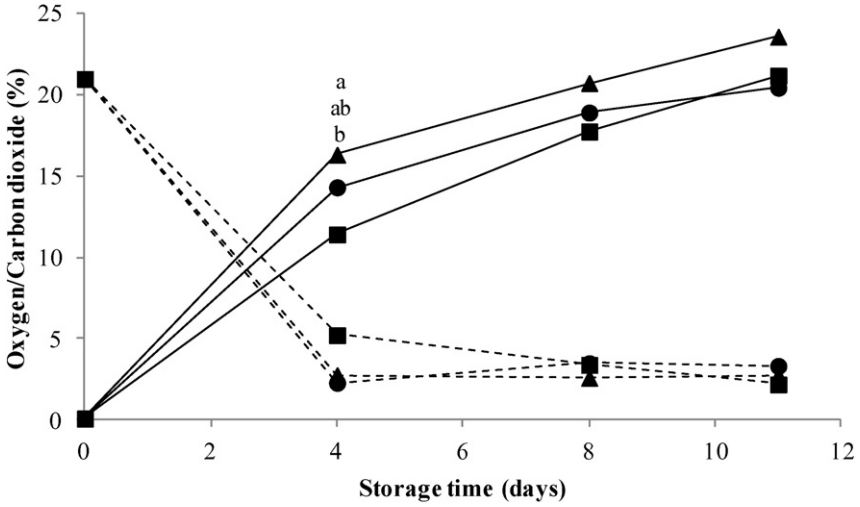

Fig. 2. Atmosphere changes of O_2 (dashed lines) and CO_2 (continuous lines) inside packages of fresh-cut melon pieces untreated (circle) or inoculated with *L. plantarum* B2 (triangle), *L. fermentum* PBCC11.5 (square), and stored for 11 days at 5 °C. Reported values are means of three replicates for each sampling time. Means with different letters at the same time of storage are significantly different according to Tukey's test (*P* value ≤ 0.05).

L. plantarum and *L. fermentum* were plated on MRS agar after incubation at 37 °C for 48 h. The level of *L. monocytogenes* contamination was determined after growth on PALCAM agar plates incubated at 37 °C for 48 h.

3. Results and discussion

3.1. Viability of probiotic LAB on fresh-cut cantaloupe

In this work, the riboflavin over-producing strains *L. plantarum* B2 and *L. fermentum* PBCC11.5, previously characterized for their probiotic features by using *in vitro* and *in vivo* models (Arena et al., 2014; Russo et al., 2015), were inoculated on fresh-cut cantaloupes in order to obtain a new functional food. With a similar approach, the same LAB strains were suggested for the preparation of probiotic fresh-cut pineapples (Russo, de Chiara, et al., 2014). Nonetheless, the present study could be of interest in order to extend the concept of probiotic fortification also to minimally processed low-acid fruits.

In order to make this innovation attractive for industrial applications, the inoculum of high amount of probiotics should be fast, inexpensive and scalable. A typical step in the production of minimally

Table 1
Effect of dipping treatment in probiotic-enriched solutions, time of storage and treatment × time of storage on fresh-cut melon qualitative attributes. Mean values of 9 samples (3 replicates × 3 storage times).

	Control	*L. fermentum* PBCC11.5	*L. plantarum* B2	A: storage time	B: treatment	Interaction A × B
Chemical attributes						
Ascorbic acid (mg/100 g)	27.24	24.67	25.25	**	ns	**
Dehydroascorbic acid (mg/100 g)	2.29	1.78	1.68	**	ns	**
Vitamin C (mg/100 g)	29.53	26.45	26.93	***	ns	*
Total phenols (mg gallic acid/100 g)	23.31	24.84	21.10	ns	ns	ns
Antioxidant Capacity (mg Trolox eq/100 g)	17.28	16.85	19.66	ns	ns	ns
Sucrose (mg/100 g)	61.57	63.39	59.13	ns	ns	ns
Glucose (mg/100 g)	10.30b	8.59c	13.37a	****	****	***
Fructose (mg/100 g)	4.84b	4.31c	6.12a	***	****	****
Citric acid (mg/100 g)	2.46b	2.60b	2.18a	****	*	***
Malic acid (mg/100 g)	1.36b	1.33b	1.68a	****	****	****
Fumaric acid (mg/100 g)	0.06a	0.08a	0.05b	***	***	ns
Ethanol (μmol/g)	0.44b	0.42b	0.53a	****	**	*
Acetaldehyde (μmol/g)	0.13	0.12	0.15	*	ns	**
Sensorial attributes						
Odor	3.39	3.20	2.96	****	ns	ns
Off odor	1.22b	1.28b	2.22a	****	****	****
Appearance	3.31	3.37	3.24	****	ns	ns
Color	3.30	3.43	3.24	****	ns	ns
Firmness	3.24	3.41	3.26	****	ns	ns
Herbaceous	1.91	1.86	1.91	****	ns	ns
Sweetness	3.69	3.72	3.65	ns	ns	ns
Off flavor	1.22b	1.17b	1.78a	**	****	ns
Color attributes						
L^*	59.22a	58.68ab	57.82b	**	**	ns
a^*	17.48b	18.08a	18.06a	*	*	ns
b^*	26.39	26.32	26.87	****	ns	ns
Chroma	31.69	31.97	32.38	***	ns	ns
Hue angle	56.32	55.32	56.05	****	ns	ns
ΔE	3.12	3.11	1.99	ns	ns	ns

Mean values followed by different letter(s), are significantly different (P ˂ 0.05) according to Tukey test. (****) P ≤ 0.0001; (***) P ≤ 0.001; (**) P ≤ 0.01; (*) P ≤ 0.05; ns, not significant.

processed fruits is their washing in a dipping solution containing antibrowning agents, antimicrobials, and texture preservatives. As previously suggested by Russo, de Chiara, et al. (2014), this solution could be effortlessly enriched with probiotic bacteria in a way that could provide an efficient transfer of microorganisms to the surface of minimally processed fruits.

As reported in Fig. 1, dipping of fresh-cut cantaloupes in a solution containing 1×10^{10} CFU mL^{-1} of probiotics resulted in a contamination of the food approximately two order of magnitude lower. Although, the initial level of the inoculum was compatible with the elaboration of a new functional food, a microbial viability higher than 10^6 CFU g^{-1} should be ensured at the time of consumption. Therefore, the viability of both *L. plantarum* B2 and *L. fermentum* PBCC11.5 was monitored during 11 days of storage at refrigeration temperatures. Interestingly, *L. plantarum* B2 population was almost constant over the time and maintained a concentration of about 3×10^8 CFU g^{-1} at the end of the shelf life (Fig. 1A). In contrast, a slight reduction of *L. fermentum* PBCC11.5 was observed with a final level of 7.8×10^7 CFU g^{-1} (Fig. 1A).These findings indicated that at the time of consumption cantaloupes contained more viable probiotics than the minimum level recommended to provide health benefits (Tripathi & Giri, 2014). This means that taking into account an approximate intake of 150 g serving of food, probiotic cantaloupes could deliver about 10^{10} viable cells into the intestine. Moreover, it should be considered that standard methodology for total plate counts tends to underestimate the cell numbers of these products since microbial cells in foods are exposed to a number of stressful conditions during food processing and formulation (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011).

From a microbiological point of view, the addition of probiotic LAB did not significantly affect the dynamic populations of mesophilic microorganisms, yeasts and molds (Fig. 1B;C), according to what previously reported for other minimally processed fruits (Alegre et al., 2011; Rößle et al., 2010; Russo, de Chiara, et al., 2014).

3.2. Physico-chemical analysis

The main physico-chemical features of fresh-cut cantaloupes were monitored in order to determine if the probiotic enrichment could negatively affect some organoleptic, nutritional or sensorial quality attributes of the product.

Concerning oxygen and carbon dioxide evolution inside the bags, slight differences were found between treated and control samples (Fig. 2). After 4 days of storage, samples inoculated with *L. plantarum* B2 showed a higher CO_2 percentage compared to the other melon samples. Subsequently, carbon dioxide evolution performed similarly for all treatments. Oxygen concentration dropped after four days of storage and remained quite stable around values of approximately 3% up to the last day of storage without reaching anaerobic conditions within

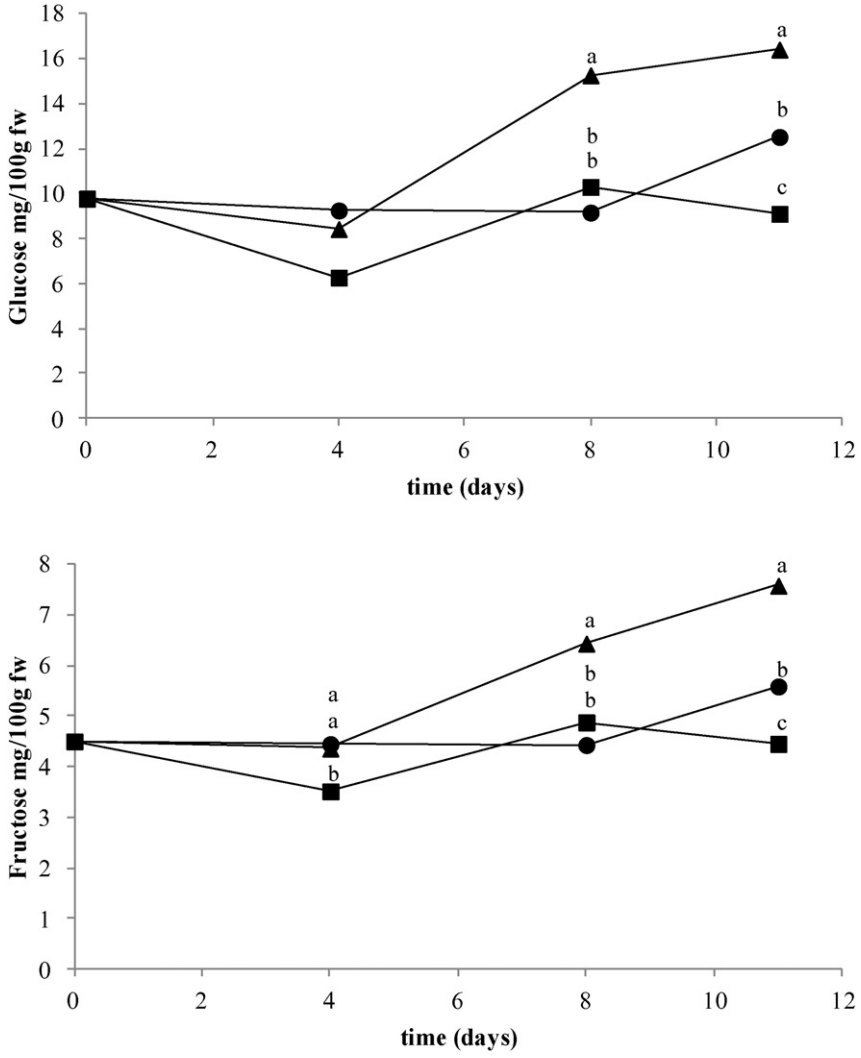

Fig. 3. Glucose and fructose (mg/100g of fresh weight (fw)) evolution of fresh-cut melon pieces untreated (circle) or inoculated with *L. plantarum* B2 (triangle), *L. fermentum* PBCC11.5 (square), and stored for 11 days at 5 °C. Reported values are means of three replicates for each sampling time. Means with different letters at the same time of storage are significantly different according to Tukey's test (P value ≤ 0.05).

the packages. No effects of *L. fermentum* PBCC11.5 or *L. plantarum* B2 inoculation on gas changes within the packages of minimally processed melons were observed.

Table 1 reports the effect of storage time, treatment and their interaction on the monitored traits for fresh-cut melon. Probiotic bacteria and storage time showed no effect on total phenols content and antioxidant capacity. These parameters remained fairly stable during storage. Similarly, sucrose content, sweetness and global color variation (ΔE) resulted to be not significantly influenced by storage time and treatment.

Ascorbic acid showed for uninoculated samples and *L. fermentum* PBCC11.5 inoculated samples a slight increase during storage time. On the contrary the treatment with *L. plantarum* B2 caused a decrease of this compound in fresh-cut melon. At the end of the storage ascorbic acid and dehydroascorbic acid content of *L. plantarum* inoculated samples resulted to be respectively lower and higher than the control, showing a greater degree of ascorbic acid oxidation. As a result, vitamin C content after 8 days of storage, was different among the three samples, resulting significantly higher for control sample (data not shown).

Fig. 3 shows glucose and fructose evolution during storage time. It was observed that melon pieces inoculated with *L. plantarum* B2 showed a significant higher content for these sugars, probably due to sucrose metabolism of the probiotic bacteria (Gänzle, Vermeulen, & Vogel, 2007). Sucrose content, in fact, slightly decreased starting from 65.27 mg per 100 g of fresh weight, to 59.61 for this sample. On the other side, *L. fermentum*PBCC11.5-inoculated melon pieces and uninoculated sample showed sucrose values of 63.08 and 60.45 mg/100 g, respectively. Melon pieces dipped in *L. fermentum* PBCC11.5-enriched solution implies a more stable trend of these compounds over time, showing no sucrose reduction during storage time. This sucrose reduction activity of *L. plantarum* B2 was also observed on probiotic enriched fresh-cut pineapples (Russo, de Chiara, et al., 2014). However, solid sugar content remained fairly stable for all samples up to 11th day, ranging from 9.8 and 10.6 °Brix without differences between the samples.

Concerning sensory attributes, most of them were not affected by probiotic treatment. After 11 days of storage, the monitored parameters (i.e. odor, appearance, color, firmness, herbaceous and sweetness) were similar for all the samples (Fig. 4). Inoculation with high concentration of probiotic bacteria did not affect the quality of fresh-cut melon. Moreover, at the end of the experiment, melon pieces showed appearance score equal to 3 corresponding to the limit of marketability, with no differences due to the treatment. It is therefore possible to state that dipping in probiotic-enriched solution did not affect fresh-cut melon visual quality. However, treatment with probiotic solution containing *L. plantarum* B2 affected in a significant way off odor and off flavor, and Fig. 5 shows their evolution over time. These findings indicated that the addition of *L. plantarum* B2 impaired some sensorial attributes of fresh-cut cantaloupes, probably due to a more intense metabolic activity as supported by the higher O_2 and sucrose consumption rates. It is well known that LAB proliferation can provoke negative perception of the organoleptic properties of fresh-cut vegetables due to the production of CO_2, ethanol, organic acids and volatile esters (Jacxsens, Devlieghere, Ragaert, Vanneste, & Debevere, 2003). However, in contrast to these results no differences were observed in the trends of off-flavor and off-odor when *L. plantarum* B2 was inoculated on fresh-cut pineapples (Russo, de Chiara, et al., 2014). This different behavior suggested that the sensorial quality of low-acidic fruit could be compromised more easily by the metabolic activity of probiotic LAB. However, up to the eighth day of storage off-flavor and off-odor were perceived within the limits of acceptability, suggesting that a short shelf life should not noticeably alter the sensory quality of the product.

Color parameters were little influenced by storage time rather than treatment type. However, uninoculated control samples maintained higher lightness and lower a^* values up to the end of storage time, showing a slightly smaller loss of brightness and color changes compared to the probiotic treated samples (data not shown). In any case, minimal color variation is often observed during storage of minimally processed cantaloupe melons (Saftner, Bai, Abbott, & Lee, 2003) but they do not affect or limit quality of fresh-cut melons (Amaro, Beaulieu, Grimm, Stein, & Almeida, 2012), in fact, sensory evaluation of melon pieces color by panelists gave similar results for all the samples, without significant differences (Fig. 4).

3.3. Riboflavin fortification

From a nutritional point of view, cantaloupe provides a wide variety of antioxidant and anti-inflammatory phytonutrients, and it is a good source of dietary fiber, vitamin A, vitamin C, and some B-group vitamins. However, riboflavin is present only in low amounts. Therefore, in this study it was investigated if contamination with riboflavin overproducing LAB strains could be a valuable strategy to further increase the nutritional value of fresh-cut melons. With this aim, the riboflavin content of cantaloupe samples was analytically monitored throughout the shelf life. Melons had an initial amount of vitamin B_2 corresponding to 6.5 μg per 100 g of edible product (Fig. 6). This concentration was constant during the first week of storage, and then came down by about 15% at the end of the shelf life. Interestingly, the riboflavin content of samples inoculated with *L. plantarum* B2 increased by 33% after four days of storage, and doubled at the end of the shelf life achieving a final concentration of about 12 μg per 100 g (Fig. 6). A similar pattern was observed when *L. fermentum* PBCC11.5 was artificially inoculated on cantaloupe pieces, although the final amount of vitamin B2 was approximately 10 μg per 100 g (Fig. 6).This agree with previous results that showed that *L. plantarum* B2 was able to produce about two-fold more riboflavin than *L. fermentum* PBCC11.5 when inoculated in a synthetic media (Arena et al., 2014; Russo, Capozzi, et al., 2014). In addition, both probiotics were able to overproduce riboflavin in co-culture with human Caco-2 cell lines (Arena et al., 2014), and to colonize the gut of gnotobiotic zebrafish larvae (Russo et al., 2015). These evidences suggested that, if carried at intestinal level through fortified foods, these microorganisms could contribute to increase the riboflavin supply in the gut environment.

3.4. Biocontrol of L. monocytogenes

Fresh-cut fruits can be a vehicle of several pathogens, including *L. monocytogenes*. Among the green strategy to fight foodborne

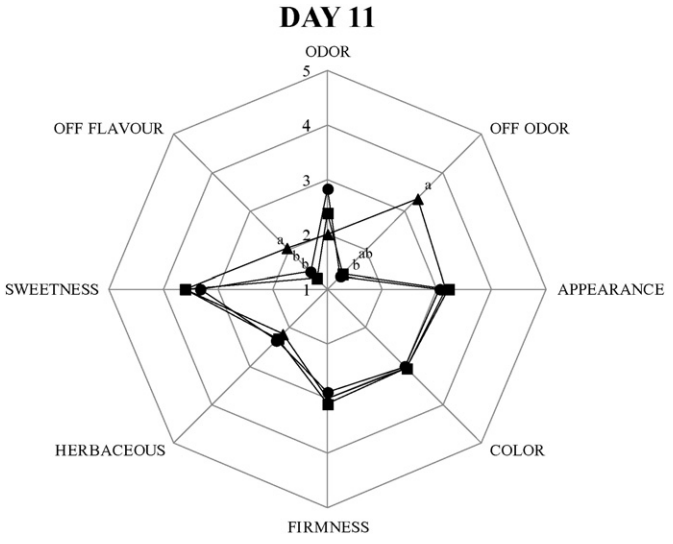

Fig. 4. Sensory properties of fresh-cut melon pieces inoculated with *L. plantarum* B2 (triangle), *L. fermentum* PBCC11.5 (square), or not inoculated (circle) and stored for 8 days at 5 °C. Reported values are means of three replicates for each sampling time and they are expressed by using a hedonic scale from 1 to 5 (1 = not present/very low/not typical and 5 = very pronounced/very typical). Means with different letters are significantly different according to Tukey test (P value ≤ 0.05).

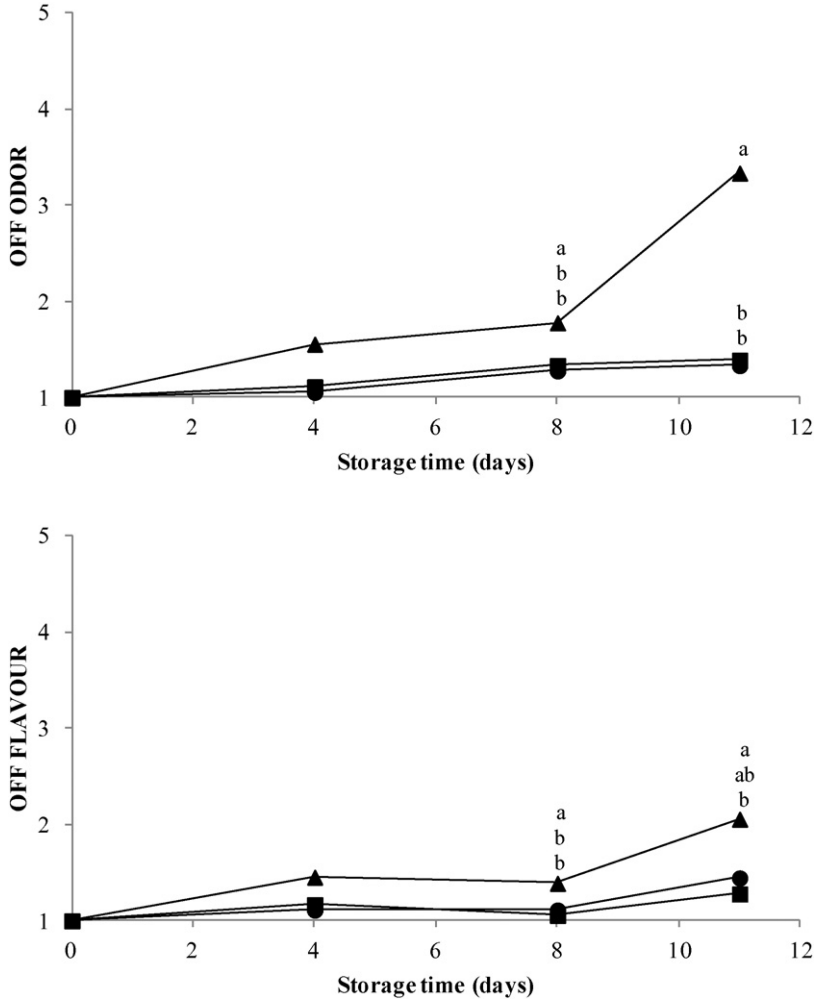

Fig. 5. Sensory parameters evolution of fresh-cut melon pieces untreated (circle) or inoculated with *L. plantarum* B2 (triangle), *L. fermentum* PBCC11.5 (square), and stored for 11 days at 5 °C. Reported values are means of three replicates for each sampling time. Means with different letters at the same time of storage are significantly different according to Tukey's test (P value ≤ 0.05).

pathogenic microorganisms, lactic acid bacteria have been proposed as protective cultures for minimally processed fruits and vegetables (Rodgers, 2008; Siroli et al., 2015). In a previous work, it was observed that *L. plantarum* B2 and *L. fermentum* PBCC11.5 were able to partially inhibit the growth of *L. monocytogenes* serotype 1/2a and *E. coli* O157:H7 on fresh-cut pineapples (Russo, de Chiara, et al., 2014). In a similar way, in this study the effectiveness of both *L. plantarum* B2 and *L. fermentum* PBCC11.5 as bioprotective agents was tested in fresh-cut

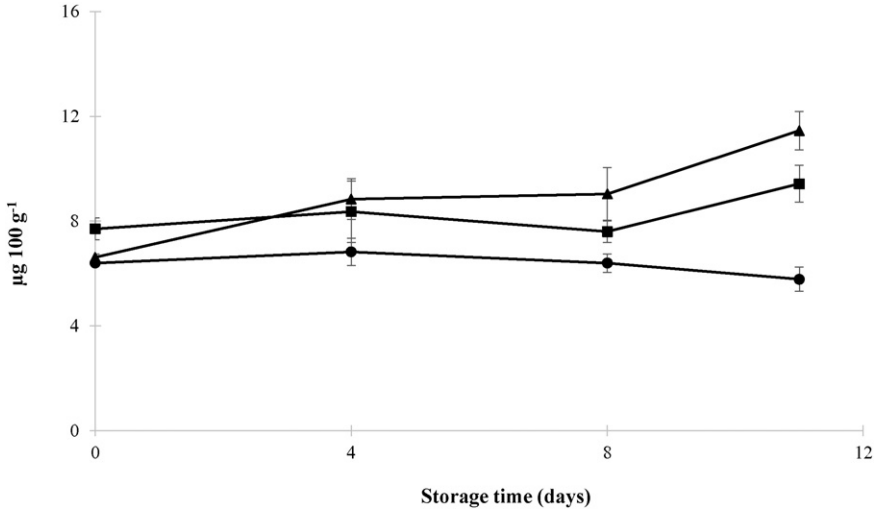

Fig. 6. Riboflavin concentration of fresh-cut cantaloupe not inoculated (circle), or inoculated with *L. plantarum* B2 (triangle), and *L. fermentum* PBCC11.5 (square) after 0, 4, 8, and 11 days of storage at 4 °C.

cantaloupes against *L. monocytogenes* A.9.4 serotype 4b. A number of studies reported that lineage II (including serotype 1/2a) isolates of *L. monocytogenes* are usually more frequently recovered from foods and food plant environments as compared to lineage I isolates (including s erotype 4b) (reviewed by Orsi, den Bakker, & Wiedmann, 2011). However, in the present work a 4b isolate from strawberry origin was selected for the antagonistic assay, since the source of isolation could be related to an increased ability of the strain to grow and survive in fruits and fruit-associated environments. Moreover, Buncic, Avery, Rocourt, and Dimitrijevic (2001) showed that the virulence of 1/2a

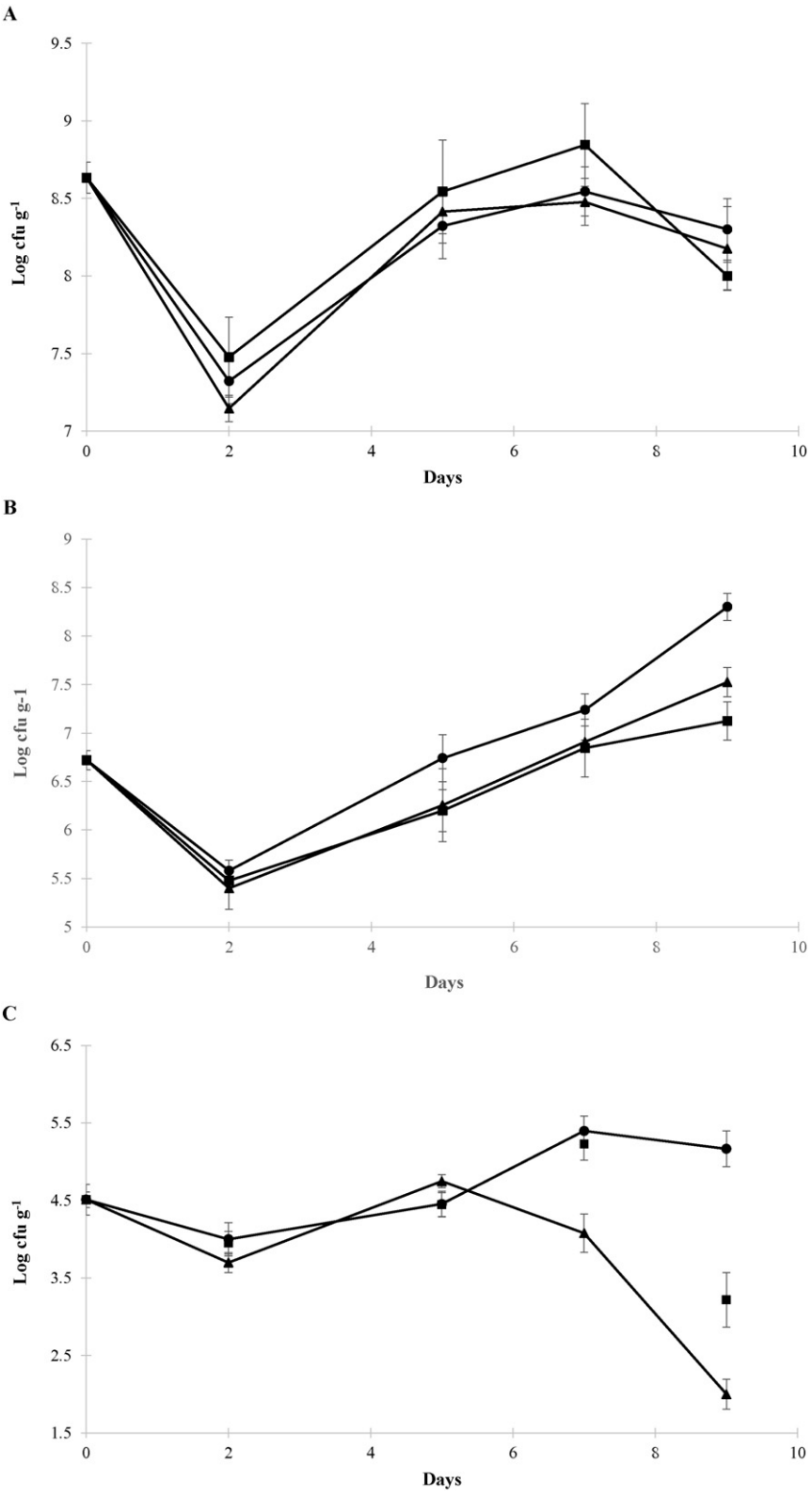

Fig. 7. Viability of *L. monocytogenes* A.9.4 artificially inoculated on fresh-cut cantaloupe at a concentration of 5×10^8 CFU g^{-1} (A),5×10^6 CFU g^{-1}(B), 5×10^4 CFU g^{-1}(C). The pathogen was inoculated without probiotics (circle), or co-inoculated with *L. plantarum* B2 (triangle), and *L. fermentum* PBCC11.5 (square).

isolates decreased after storage at 4 °C, whereas the virulence of 4b isolates remained unchanged, suggesting an improved ability to cause disease of serotype 4b strains in foods that are stored under refrigeration temperatures.

Recently, the growth rate of *L. monocytogenes* on fresh-cut melons was predicted as a function of temperature and time storage by a mathematical model (Danyluk, Friedrich, & Schaffner, 2014). However, experimental evidences showed that *L. monocytogenes* was able to growth from 10^2 to 10^9 CFU g^{-1} in melon pulp after 7 days of storage at 10 °C (Penteado & Leitão, 2004). Similarly, *L. monocytogenes* population increased on apple flesh at both 5 and 10 °C (Alegre et al., 2011), suggesting that higher levels of contamination could occur even if the cold chain is properly respected. Furthermore, it was reported that the antagonistic effectiveness of *Pseudomonas graminis* CPA-7 on fresh-cut melon was strictly related to the concentration of the pathogen (Abadias et al., 2014).

Therefore, the antagonistic effect of *L. plantarum* B2 and *L. fermentum* PBCC11.5 was tested by using three different levels of contamination of *L. monocytogenes*. In particular, when *L. monocytogenes* was inoculated at the same concentration of the probiotic bacteria (about 10^8 CFU g^{-1}), the population of the pathogen remained constant during the storage and the growth was unaffected by co-inoculation with both probiotic strains (Fig. 7A). If melons were contaminated with approximately 5×10^6 CFU g^{-1} of *L. monocytogenes*, the pathogen was able to increase up to 3×10^8 CFU g^{-1} at the refrigeration conditions (Fig. 7B). However, the growth diminished of about 1-log if also the probiotic were inoculated (Fig. 7B). A contamination of 5×10^4 CFU g^{-1} resulted in a slight increase of the pathogen that reached a final concentration of 10^5 CFU g^{-1} in control samples. Interestingly, the *L. monocytogenes* population dropped of about two- and three-log of CFU g^{-1} in melons co-inoculated with *L. fermentum* and *L. plantarum*, respectively (Fig. 7C). In contrast, *L. fermentum* PBCC11.5 seemed more effective than *L. plantarum* B2 against *L. monocytogenes* CECT 4031 (Russo, de Chiara, et al., 2014), suggesting that several factors as well as serovar, source of isolation, and food substrate could influence the potential of probiotic LAB as bioprotective agents.

4. Concluding remarks

In this study, a microbial approach was proposed to produce multifunctional fresh-cut cantaloupe. In particular, our results showed that the addition of probiotic riboflavin over-producing LAB strains could be a valuable strategy to improve the quality of fresh-cut cantaloupe at three different levels. For the first time, minimally processed melons have been suggested as carrier of beneficial microorganisms for the preparation of a new functional food. Secondly, the probiotic strains were able to *in situ* fortify the riboflavin content of fresh-cut cantaloupes, thus increasing the nutritional value of this product. Finally, the probiotic LAB could enhance the safety of minimally processed melons due to their antagonistic effect against on isolate of *L. monocytogenes* from fruit origin. However, the addition of *L. plantarum* B2 resulted in a worsening of some sensorial attributes after 11 days of storage. This finding suggested that a careful selection of probiotic strains should be always recommended in order to avoid technological changes due to the inoculated probiotics. Alternatively, a short shelf life or different technologies to deliver probiotic microorganisms, as well as encapsulation, might be considered. Therefore, further insights in the field of functional fresh-cut fruits should be encouraged with the aim to ensure high qualitative standard of these foods.

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