



Inclusion of prebiotics on the viability of a commercial *Lactobacillus casei* subsp. *rhamnosus* culture in a tropical fruit beverage

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Revised: 16 December 2018 / Accepted: 26 December 2018 / Published online: 4 February 2019
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Abstract The viability of probiotics in the development of functional tropical fruit beverages is a technological challenge that may benefit from the addition of prebiotics, due to the synergistic (synbiotic) interaction. This study evaluated the viability of a commercial probiotic (*Lactobacillus casei*) in a blended red fruit beverage (RFB; 20% strawberry, 10% blackberry and 5% papaya), enriched with three separate prebiotics: inulin (IN), fructooligosaccharides (FOS) and galactooligosaccharides, added at 1 and 5%. The consumer preference for the beverages was also examined. The inoculum was produced in MRS broth supplemented with 10% RFB, which reached the exponential phase ($9.96 \log \text{CFU mL}^{-1}$) after incubation at 37 °C for 24 h. In search of the probiotic strain's adaptation and viability in the presence of the different prebiotics (measured by optical density, $\text{OD}_{600 \text{ nm}}$), the prebiotics were added to MRS broth at 1 and 5%. Since 1% IN ($\text{OD} = 3.99 \pm 0.36$) and 1% FOS ($\text{OD} = 3.48 \pm 0.28$) were the most significant, these treatments, without inoculation of probiotics, were assessed by the sensory panel. Although neither RFB was significantly preferred the RFB with 1% IN received the greatest number of responses ($n = 33/60$). Its effect on the viability of *L. casei* inoculated in the RFB was monitored by the growth kinetics at 37 °C for 50 h. The findings indicated that fortification with 1% IN could have a possible protective effect on the stability of *L. casei* in RFBs, highlighting the use of tropical fruits as potential carriers of probiotics.

Keywords Prebiotic · *Lactobacillus casei* · Inulin · Red fruit · Fruit beverage

Introduction

Extensive research and development activity are currently centered around food products formulated with biologically active compounds (Mylona et al. 2018). Products with natural functionality, such as Oriental and African fermented products of vegetable origin (e.g., kimchi), for instance, are particularly attractive, owing to the broad appeal among modern consumers for “all natural”, fruit/vegetable nutrition and functional products that help promote health (El Sheikha and Montet 2014; Ray et al. 2014).

Beverages are an excellent vehicle for supplying bioactive compounds, such as vitamins, minerals, antioxidants, fiber, prebiotics and probiotics (Corbo et al. 2014), and represent one of the fastest growing markets for product development. Fruit and vegetable juices are among the most rapidly advancing sectors in the beverages industry, due to their convenience and associated health benefits. In 2016, the global fruit and vegetable juices market size was valued at USD 154.18 billion and is expected to grow at a compound annual growth rate of 5.93% during 2018–2025 (Grand View Research 2018).

A beverage containing mixtures of red berries (strawberry and blackberry) could be an attractive option for consumers, as products derived from these fruits are a significant source of bioactive components, such as polyphenols, flavonoids, ellagic acid, tannins, ellagitannins, gallic acid, benzoic acid and anthocyanins (Gaurav and Tiwari 2017; Yahia 2018). Some interesting sources of these compounds are Colombian tropical fruits, as many of

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these fruits present a high phenolic content (1011–1018 mg gallic acid equivalents 100 g^{-1} fresh weight for Brazilian guava, guava apple, banana passion fruit and cashew, for example) and antioxidant activity (Yahia 2018).

Likewise, these blended products have been proposed as novel and appropriate vehicles for the inclusion of probiotic strains because of their essential micronutrient content, allowing to produce lactic acid and biomass under fermentative conditions (Perricone et al. 2015; Yoon et al. 2005). At the same time, lactic acid fermentation of fruits and vegetables enhances their organoleptic and nutritional quality and retains the nutrients and colored pigments (El Sheikha 2018a, b). In addition, fruit-based beverages have a healthy image and present a wide variety of sensory profiles (Pereira et al. 2011; Sheehan et al. 2007).

Probiotics are non-pathogenic microorganisms that when consumed in adequate amounts, confer a health benefit on the host (Hill et al. 2014). Although probiotics have traditionally been consumed in fermented dairy products, like yogurt, the consumption of these products is limited by the rise in various diet types (e.g., lactose-free, reduced-cholesterol), leading to the development of non-dairy alternatives. In this context, inclusion matrices based on vegetables and fruits are a promising healthy option, aimed at consumers allergic to dairy products, as well as vegetarians (Corbo et al. 2014).

The fruits most commonly used in commercial juice preparations inoculated with probiotics include cranberry, apple, blackcurrant, cherry, guarana, mango, grapes, kiwifruit, strawberries, peaches and plums, and some of the typical probiotic strains used in formulating new products are *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. rhamnosus* and *Bifidobacterium lactis* (Martins et al. 2013). However, maintaining the recommended viability of probiotics in juices is technologically challenging, mainly because of the presence of insufficient amounts of peptides and free amino acids required for the metabolism of probiotic strains, as well as the susceptibility of probiotics to extreme acidic conditions (pH 2.5–3.6), and the potential for additional undesired fermentation and alterations to the sensory characteristics of food products, which may lead to poor microbiological quality and low consumer acceptability (Perricone et al. 2015; Ray et al. 2014). It is important to adapt probiotic bacteria to these kinds of products, for large-scale industrial production and processing, and that they maintain good activity during storage (El Sheikha 2018a, b).

The addition of prebiotics, substances capable of exerting a protective effect and selectively stimulating the growth of probiotics in the human intestine, is a strategy in the design of probiotic juices (Perricone et al. 2015; Shori 2016). The inclusion of prebiotics in beverages fulfills

multiple functions beyond the probiotic strains' survival, such as the improved sensory and physicochemical characteristics of the drinks, as sweetening agents for fruits drinks, and as stabilizers to avoid liquefaction processes (Munir et al. 2016). Inulin (IN), fructooligosaccharides (FOS) and galactooligosaccharides (GOS) are well-recognized prebiotics found naturally in fruits, vegetables, milk, honey and bamboo, among various other foods (Singh et al. 2015). Mixtures of probiotics and prebiotics (synbiotics) are often used in functional foods to exploit their synergistic interactions (Al-Sheraji et al. 2013). The consumption of synbiotic functional food products/beverages is a current global trend (Shori 2016).

Despite the numerous reports about the use of fruits to formulate functional beverages, studies focusing on the innovation of juices based on tropical and Andean mixtures of red fruits containing probiotic commercial lactobacilli strain and prebiotics, such as IN, are still scarce. Therefore, this research determined the influence of the addition of IN, FOS and GOS on the viability of a commercial probiotic strain (*L. casei*) in a red fruit beverage (RFB).

Materials and methods

Raw materials, culture medium and commercial prebiotics

Commercially-mature blackberry, strawberry and papaya fruits were purchased from a local market. The fruits were transformed into pulps and stored at $-14\text{ }^{\circ}\text{C}$ before the formulation of the beverage. Culture medium and broth (de Man–Rogosa–Sharpe, MRS; Oxoid[®]) were prepared according to the manufacturer's instructions. The prebiotic fibers, namely, FOS (96.4% purity; Nutraflora[®], Ingredient), IN [Orafti[®] HSI (high soluble IN powder) with 88% IN] and GOS [57% minimum GOS content (solids basis); Bioigo[™], Ingredient] were purchased, as indicated. For the preparation of the blended fruit beverage, sucrose (food grade) was used.

Formulation and production of the red fruit beverage (RFB)

The RFBs were prepared by blending 20% (w/v) of strawberry (*Fragaria × ananassa*), 10% (w/v) blackberries (*Rubus glaucus* Benth) and 5% (w/v) papaya (*Carica papaya*) pulps. Papaya was chosen as a pH stabilizer and additional fiber source ($1.7\text{ g }100\text{ g}^{-1}$) (Braga and Conti-Silva 2015). The pH was 3.36 ± 0.02 , and the total soluble solids were $10 \pm 0.01\text{ }^{\circ}\text{Brix}$. The beverages were poured into Schott bottles (250 mL) and sterilized ($120\text{ }^{\circ}\text{C}$, 15 psi, 15 min) before inoculation, to

avoid the interaction of the probiotic with the microbiota of the native fruits, which could lead to possible alterations in the beverage (Sheehan et al. 2007). The inoculation was done at 18–20 °C under aseptic conditions. All procedures were carried out in compliance with good manufacturing practices (Ministerio de Salud y Protección Social de Colombia 2013).

Culture and growth conditions

A commercial lyophilized probiotic culture of *L. casei* subsp. *rhamnosus* (Mediterranea Biotecnologie[®], Termoli, Italy) was used. The strain was activated by rehydration in 5 mL of MRS broth at 37 °C for 72 h, and the purity was checked by Gram staining.

Production of inoculum in a synthetic medium supplemented with a red fruit beverage (RFB): adaptation of the strain

For the inoculum standardization, the time of maximum biomass production of the probiotic microorganism in the synthetic medium, which simulated the formulation of the RFBs, was used as a reference. The synthetic medium consisted of MRS broth supplemented with 10% RFB, as recommended by Perricone et al. (2014). The strain was active in MRS broth at 37 °C for 72 h. Afterward, 2% of the pre-inoculum was added to 50 mL of synthetic medium. Cells were centrifuged (4000 rpm, 10 min) and suspended in 10 mL of sterile saline solution.

Evaluation of the effect of the prebiotics on the survival of *Lactobacillus casei* in modified de Man–Rogosa–Sharpe (MRS)

The prebiotics viability was evaluated in an MRS supplemented with 5% sucrose (which was added to the beverage) and the prebiotic substances: IN, FOS and GOS. The culture broth was modified to simulate the conditions of the RFB, and the viability of the *L. casei* strain in the presence of the prebiotics was assessed. Each treatment (20 mL) was inoculated with 2% (400 mL) ($7\text{--}8 \log \text{CFU mL}^{-1}$) of the studied microorganism. The controls included an MRS broth without the addition of prebiotic, and an MRS supplemented with 5% sucrose. Biomass was determined at 0, 10, 24 and 48 h of incubation at 37 °C, by measuring the optical density at 600 nm ($\text{OD}_{600\text{nm}}$) (Adebola et al. 2014). Triplicate analyses were undertaken.

Sensory analysis of the red fruit beverages (RFBs) incorporated with prebiotics

Treatments (1% IN and 1% FOS) were selected according to the results obtained from the analysis of the prebiotics in the modified MRS. The RFBs were refrigerated (4–8 °C, for no longer than 2 days) until the development of the test. Beverages were not inoculated with probiotic bacteria. A preference test (paired comparison) was conducted with a panel of 60 untrained male and female evaluators, aged between 19 and 60 years. Two treatments, namely, RFBs with 1% IN and 1% FOS, were evaluated. Samples (7–10 °C) were provided to the panelists as 10 mL of non-inoculated RFB coded with three random digits, and the test was performed as described elsewhere (Nuñez Hinojosa and Brumovsky 2010).

Survival of *Lactobacillus casei* in the red fruit beverages (RFBs): growth kinetics

Treatment with 1% IN was selected according to the viability assays in the modified MRS broth and sensorial preference. Growth kinetics were assayed in the RFBs containing 1% IN (200 mL) and inoculated with 2% (4 mL) of *L. casei* from the inoculum designed in the MRS supplemented with 10% of the RFB. Samples were incubated at 37 °C for 50 h. The number (CFU mL^{-1}) of lactobacilli was determined on MRS agar at 0, 10, 24 and 50 h. In addition, physicochemical parameters, including acidity and pH (AOAC 942.15) and the total soluble solids ($^{\circ}\text{Brix}$) (AOAC 932.12) were measured (AOAC 2012). The controls included an RFB without IN and an uninoculated RFB. Triplicate analyses were conducted.

Statistical analysis

For the interpretation of the results of the preference tests, the table provided by Rosesler et al. (1978), reprinted in Stone and Sidel (1993), was used. Analyses of the prebiotic evaluation assays and growth kinetics in RFBs with 1% IN were conducted using a generalized model test in the Minitab[®] statistical package. For each parameter evaluated (viability and physicochemical measures), the mean ($n = 3$) and standard deviation were calculated.

Results and discussion

Formulation and production of the red fruit beverage (RFB)

In the development of food with probiotic microorganisms, factors, such as the bacterial strain, inoculum preparation

method, physiological state of the cells, oxygen levels and presence of prebiotics, are considered (Vandenplas et al. 2015). These products should maintain the survival of microorganisms at levels between 10^6 and 10^7 CFU mL⁻¹ over the product's shelf life. Therefore, considering its effectiveness as a vehicle for *L. casei*, the RFB was formulated to maintain a minimum cell density of 10^6 CFU mL⁻¹, without altering the sensorial characteristics of the product. To achieve this, the beverage formulation was established with a pH around 3.3. Studies on RFBs with probiotics show that the composition of the drink and the adjustment of the pH in the final product are key to the viability of the microorganism in the product (Nematollahi et al. 2016). Therefore, it was necessary to add 5% papaya to the strawberry and blackberry mixture to stabilize the pH values, achieving average pH values of 3.36 ± 0.02 , in which the viability was maintained. A low pH may have an antagonistic effect against pathogenic microorganisms. At the same time, the total soluble solids, pH and percentage of fruits were established, in compliance with current Colombian regulations for this type of product (Resolution 3929 of the Ministry of Health and Social Protection 2013). Red fruits, such as cherries, cranberries, blackberries and raspberries, contain components with antimicrobial properties (Perricone et al. 2015; Sheehan et al. 2007; Yahia 2018). Other factors that may influence the viability of probiotics in this type of matrix are the high content of phenols, as exemplified by the low viability of *L. plantarum* in lemon, pomegranate and cranberry juices, respectively (Nualkaekul and Charalampopoulos 2011).

Production of inoculum in a synthetic medium supplemented with a red fruit beverage (RFB): adaptation of the strain

Inoculum production with adaptation and scaling of the probiotic directly into the RFB was key to adapting the microorganism to the matrix, as a fermentation substrate allowing microbiological stability (Pereira et al. 2011). The inoculum production in the synthetic medium reached the exponential phase ($9.96 \log$ CFU mL⁻¹) after incubation at 37 °C for 24 h. These conditions allowed the adaptation of *L. casei*, slightly inducing its resistance to the pH (3.36 ± 0.02) of the RFB and obtaining the highest cell density, based on the method suggested by Perricone et al. (2014).

Strain adaption proved effective in a previous study, evaluating the viability of *L. reuteri* in various fruit juices, in which the type of juice strongly affected the viability of the probiotic: surviving in pineapple, orange and apple juices while exhibiting a strong reduction in juice prepared from red fruits (Perricone et al. 2014). Supplementation with 10% of the drink in MRS significantly increased the

initial reduction time of 0.47 days out to 9.28–11.20 days (Perricone et al. 2014).

Evaluation of the effect of the prebiotics on the survival of *Lactobacillus casei* in the modified de Man–Rogosa–Sharpe (MRS) medium

Table 1 describes the viability of *L. casei* in the modified MRS broth, in the presence of the prebiotics. Significant differences ($p < 0.05$) were found between the treatments with 1 and 5% GOS, as compared to the controls (MRS broth and 5% sucrose MRS broth), with a favorable effect on the viability of *L. casei* at low levels (1%). The protective effect may be because, as reported previously, prebiotics could maintain the residual water levels necessary to preserve cell structures, preventing adverse effects on microorganisms by creating a barrier against environmental factors. Prebiotics can be considered as protective molecules (Tymczyszyn et al. 2011). For example, the microencapsulation of probiotic microorganisms with prebiotic agents has been used to increase the survival of these microorganisms in synbiotic foods (Yonekura et al. 2014). In another study, IN acted as protective agent in the formation of synbiotic microcapsules, to improve the heat resistance of probiotics (Karimi et al. 2015).

The highest absorbance value at 50 h of incubation was obtained in the treatment with 1% GOS ($OD_{600nm} = 4.02 \pm 0.11$), followed by the treatment with 1% IN ($OD_{600nm} = 3.99 \pm 0.36$) and 1% FOS ($OD_{600nm} = 3.48 \pm 0.24$). However, excessive caramelization was observed in the culture medium with the addition of GOS at both levels (1 and 5%) after sterilization, which led to disregarding this prebiotic because of its possible effect on the sensory qualities of the beverage (Braga and Conti-Silva 2015). It was observed that the treatment with 1% IN was more viable than the control with MRS ($OD_{600nm} = 3.83 \pm 0.06$), indicating that IN might have exerted a protective influence on *L. casei*, improving its survival. It has been reported that IN has more pronounced prebiotic effects than FOS under fermentative conditions (37 °C), in simulators of the human intestinal microbial ecosystem (Shoaib et al. 2016).

Sensory analysis of the red fruit beverages (RFBs) incorporated with prebiotics

Considering the results above, sensory analysis of the RFBs fortified with 1% IN and 1% FOS, respectively, without inoculation of the probiotic bacteria was performed using a preference test. Although the number of similar responses in the test indicated that there was no tacit sensory outcome on the beverage preference for the prebiotic type (Table 2, $p > 0.05$), the highest number of responses

Table 1 Effect of prebiotics on the viability of *L. casei* (DO600 nm) in an MRS broth modified with 5% sucrose

Treatment	Time (h)			
	0	10	24	50
T1: MRS Broth + 5% sucrose + 1% IN	0.08 ± 0.03 ^{ab}	0.71 ± 0.04 ^{ab}	3.43 ± 0.30 ^{ab}	3.99 ± 0.36 ^{ab}
T2I: MRS Broth + 5% sucrose + 5% IN	0.09 ± 0.01 ^c	0.75 ± 0.07 ^c	2.31 ± 0.04 ^c	3.15 ± 0.40 ^c
T3: MRS Broth + 5% sucrose + 1% FOS	0.06 ± 0.03 ^{abc}	0.82 ± 0.05 ^{abc}	2.99 ± 0.05 ^{abc}	3.48 ± 0.24 ^{abc}
T4: MRS Broth + 5% sucrose + 5% FOS	0.07 ± 0.00 ^{bc}	0.64 ± 0.03 ^{bc}	2.69 ± 0.15 ^{bc}	3.34 ± 0.34 ^{bc}
T5: MRS Broth + 5% sucrose + 1% GOS	0.06 ± 0.06 ^{abc}	0.80 ± 0.07 ^{abc}	2.42 ± 0.18 ^{abc}	4.02 ± 0.11 ^{abc}
T6: MRS Broth + 5% sucrose + 5%GOS	0.03 ± 0.03 ^{bc}	0.65 ± 0.10 ^{bc}	2.67 ± 0.20 ^{bc}	3.67 ± 0.16 ^{bc}
C1: MRS Broth	0.08 ± 0.07 ^{abc}	0.75 ± 0.03 ^{abc}	3.23 ± 0.13 ^{abc}	3.83 ± 0.06 ^{abc}
C2: MRS Broth + 5% sucrose	0.04 ± 0.01 ^a	0.86 ± 0.03 ^a	2.94 ± 0.08 ^a	4.95 ± 0.30 ^a

Each value is the mean ± standard deviation of the mean of three replications. Absorbance values with the same letter in the treatments do not differ significantly from each other at a significance level of 95% according to the Tukey test

Table 2 Sensory analysis: preference test for the prebiotics in the RF beverages

Type of beverage	Number of product acceptance responses
RF drink with 1% IN	33 ^a
RF drink with 1% FOS	27 ^a

Number of responses followed by the same letter in the rows did not differ significantly from each other at a significance level of 95% according to the statistical table of Rosesler et al. (1978), reprinted in (Stone and Sidel 1993)

was for the beverage enriched with 1% IN (Karimi et al. 2015).

Other authors have reported similar results, in which there was no evidence of preference differences between beverages with prebiotics and their traditional counterparts (Braga and Conti-Silva 2015; Cassani et al. 2017). One study examined the sensory impact of prebiotics (FOS and IN) and sugar substitutes on papaya nectars and found that the preference was similar to the nectars with sugar (Braga and Conti-Silva 2015). Additionally, in optimization processes for the formulation of strawberry juices with probiotics, the addition of IN demonstrated no negative impacts on sensory quality (Cassani et al. 2017). However, the magnitude of the effect of IN on the sensory acceptability depends on the blend of the short- and long-chain IN, as well as the total IN content (Shoab et al. 2016).

Survival of *Lactobacillus casei* in the red fruit beverages (RFBs): growth kinetics

According to the probiotic viability in the modified MRS broth, and considering that the prebiotic type did not alter the sensory response, the viability of *L. casei* on the RFB incorporated with 1% IN was selected to determine the

growth kinetics under fermentation conditions that simulate the normal temperature of the human body, i.e., 37 °C (Fig. 1). Inoculation of *L. casei* into the RFB with 1% IN and a control (RFB without 1% IN) significantly affected ($p < 0.05$) the viability and physicochemical properties of the RFB. The plate counts were 7.03 and 6.81 log CFU mL⁻¹ in the treatments with and without IN, respectively. It suggests that the addition of 1% IN may improve the viability of the probiotic microorganism. The stimulation of the metabolism of lactic acid bacteria (*Lactobacillus*) by IN may be due to partial hydrolysis and subsequent metabolization of fructose, as an additional source of carbon and energy (De Souza Oliveira et al. 2012).

Some studies on juices prepared from red fruits, like blueberries, blackcurrants and blackberries, have reported a drastic decrease in viability because of the presence of certain organic acids (benzoic acid) in red fruits that may have toxic effects on microorganisms and considerably

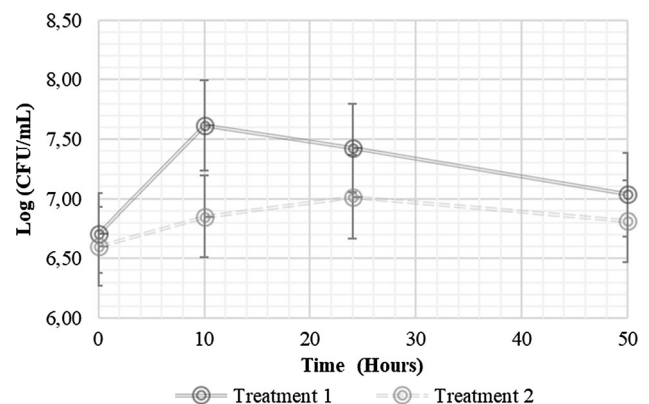


Fig. 1 Growth kinetics of *L. casei* in the RF beverages. Treatment 1: red fruit beverage with 1% IN. Treatment 2: red fruit beverage without the addition of prebiotics

affect the viability of the probiotic culture during processing and in its future useful life (Sheehan et al. 2007). When various *Lactobacillus* and *Bifidobacterium* strains were added to orange, pineapple and cranberry juices, a large difference in the acid resistance was observed, even at the same pH, and all strains survived least in the cranberry juice, possibly because of the high content of benzoic acid (34 mg L^{-1}) (Sheehan et al. 2007). Therefore, authors, such as Perricone et al. (2015), have recommended strategies for the adaptation of the strains and the use of the prebiotics, such as those used in the current investigation.

In the absence of added prebiotics, Di Cagno et al. (2011) identified lactic acid bacteria (*L. plantarum*, *Lactobacillus* sp. and *L. pentosus*) from several fruits (blackberries, prunes and raisins) and developed a protocol for processing functional smoothies (without prebiotics) with acceptable nutritional, antioxidant and sensory attributes, despite the hostile environment (pH about 3.51 and high polyphenol content). Conversely, 1% IN improved the viability of a *L. plantarum* CECT 220 in blended carrot and orange juice, presenting 9.2 and 5.8 log CFU mL⁻¹ in juices with and without the added prebiotic, respectively, and a higher monosaccharide concentration was retained relative to the juice without IN (40% lower) (Valero-Cases and Frutos 2017). The level of 1% IN is beneficial for sensory attributes and the microbiology stability of the RFB, possibly because the blend of short- and long-chain IN at 50:50 ratios provides various additional advantages in enhancing prebiotic effectiveness (Shoaib et al. 2016).

As already mentioned above, the incorporation of prebiotics into fruit juices presents several technical challenges. The compatibility of these ingredients with the products, regarding physicochemical, sensory and nutritional properties, must be well established (Fonteles and Rodrigues 2018). The current research demonstrated significant impacts of the growth of *L. casei* on the physicochemical parameters (pH, titratable acidity and soluble solids; Figs. 2, 3 and 4, respectively) of the RFBs, including the non-inoculated controls ($p < 0.05$). Decreases in pH of 3.36 ± 0.02 to 3.26 ± 0.01 occurred for both the 1% IN and no prebiotic treatment (Fig. 2), possibly because of the conversion of sugars to organic acids (e.g., malic and lactic) (Valero-Cases and Frutos 2017), and concomitant decrease in the initial pH values. Lactic acid bacterial cultures have been reported to be characterized by acid production that lowers pH and increases the acidity of the medium, creating an unfavorable environment for pathogens and altering microorganisms (Pereira et al. 2011). The acidity, although showing significant differences between the treatments with and without IN, did not exhibit marked variations in the fermentation time when compared to the non-inoculated controls (Fig. 3). Figure 4

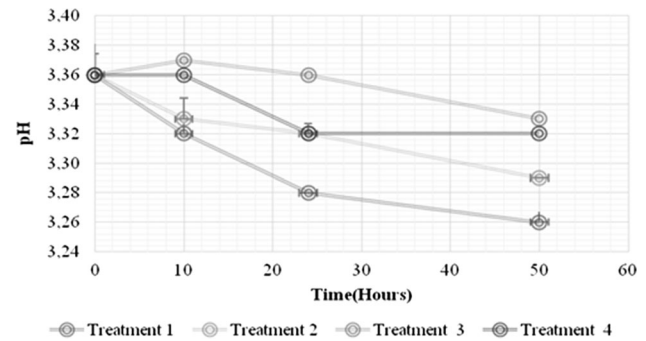


Fig. 2 Effect of the 1% IN on the pH of the growth kinetics of *L. casei* in the RF beverages. Treatment 1: RF beverage with 1% IN and *L. casei*. Treatment 2: fed fruit beverage without the addition of 1% IN with *L. casei*. Treatment 3: red fruit beverage control with 1% IN and without inoculation. Treatment 4: red fruit beverage without 1% IN and without inoculation

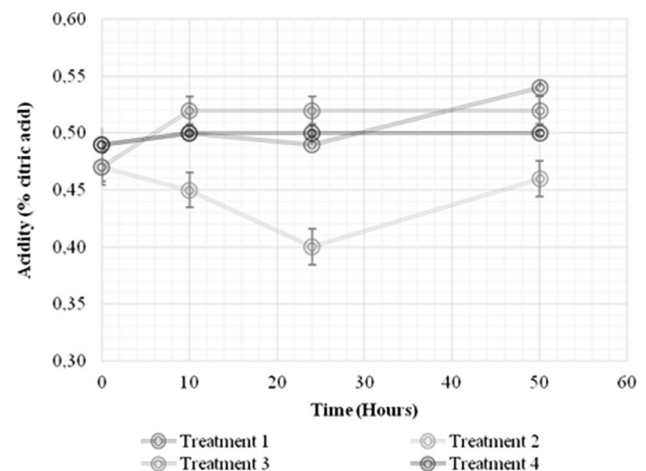


Fig. 3 Effect of 1% IN on the titratable acidity in the growth kinetics of *L. casei* in a RF beverage. Treatment 1: red fruit beverage with the addition of 1% IN and *L. casei*. Treatment 2: red fruit beverage without 1% IN and with *L. casei*. Treatment 3: red fruit beverage control with 1% IN and without inoculation. Treatment 4: red fruit beverage without 1% IN and without inoculation. The acidity is expressed as a percentage of citric acid in the sample

depicts the effect of 1% IN on the total soluble solids in the RFBs ($p < 0.05$). An increase in the soluble solids content ($^{\circ}$ Brix) of the RFBs with IN (treatments 1 and 3) was evidenced relative to those that did not contain the prebiotic (treatments 2 and 4), independent of the *L. casei* growth. It likely indicates that the main sources of carbon and energy for *L. casei* were glucose and fructose while IN was fermented relatively more slowly (Valero-Cases and Frutos 2017). Similar trends in the total soluble solids have been noted in papaya nectars with added IN (Orafti[®]P95) and FOS that were appreciated for their taste and general acceptance in equal measure as nectars containing only sugar (Braga and Conti-Silva 2015).

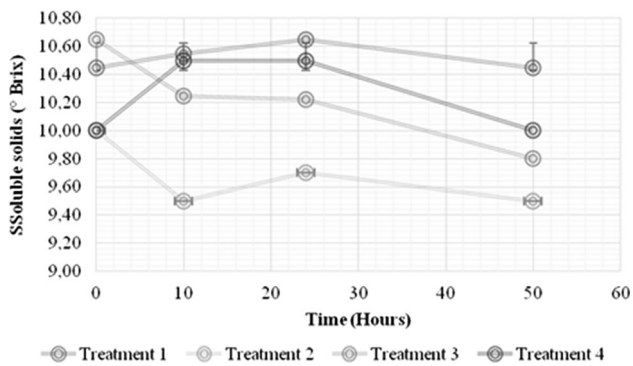


Fig. 4 Effect of 1% IN on the soluble solids in the growth kinetics of *L. casei* in a RF beverage. Treatment 1: red fruit beverage with the addition of 1% IN and *L. casei*. Treatment 2: red fruit beverage without 1% IN and with *L. casei*. Treatment 3: red fruit beverage control with 1% IN and without inoculation. Treatment 4: red fruit beverage without 1% IN and without inoculation

The inclusion and viability of a commercial-type probiotic (*L. casei*) in an RFB at 35% w/v fruit (blackberry and strawberry) was achieved while addressing several biotechnological factors. First, papaya (5% w/v) was added as a natural pH stabilizer. Second, *L. casei* was adapted to the acidic conditions by supplementing the MRS broth with the RFB (10%), for the production of the inoculum. Third, prebiotics (1% IN) were added to enhance the viability of the microorganism under fermentative conditions. This research permitted the design of a method for the formulation of an RFB-type blend of tropical origin with functional characteristics: addition of probiotic and prebiotic substances. This formulation and findings could advance the generation of fruit-based synbiotic products.

Conclusion

This study showed that red and tropical fruits from Colombia, such as strawberry, blackberry and papaya, are good fruit-derived matrices for incorporation of a commercial type probiotic (*L. casei*). Adaptation of the strain (*L. casei*) by supplementing the culture medium with the RFB (10%) to produce the inoculum and the addition of prebiotics (1% IN) were key technological strategies that could be implemented to design and formulate these functional products at an industrial scale. Moreover, IN significantly affected the viability of *L. casei* and physicochemical parameters of the RFBs under fermentative conditions, without impacting on the sensory quality of the RFBs, highlighting the suitability of this prebiotic in the final formulation of novel functional drinks prepared from red fruits.

Acknowledgements The authors express their gratitude to the project “Uso de bacterias ácido lácticas para biopreservación y

generación de nuevos productos con características probióticas”, financiada by the Dirección de Investigación, Sede Bogotá of the Universidad Nacional de Colombia; the Jardín Botánico de Bogotá, for its contribution to the Convocatoria Permanente in stimulating the research “Thomas Van Der Hammen”; and the staff of the Laboratorio de Biotecnología y Calidad Microbiología de Alimentos, and the Planta de Investigación en Procesos Vegetales of the Instituto de Ciencia y Tecnología en Alimentos (ICTA).

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