Electronic Journal of Biotechnology 42 (2019) 42–48

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research Article

Survival of microencapsulated *Lactobacillus casei* (prepared by vibration technology) in fruit juice during cold storage

Araceli Olivares [a,*], Carmen Soto [a], Eduardo Caballero [a], Claudia Altamirano [a,b]

[a] Centro Regional de Estudios en Alimentos Saludables, Av. Universidad 330, Placilla, Sector Curauma, Valparaíso, Chile
[b] Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso, Chile

A R T I C L E I N F O

Article history:
Received 3 June 2019
Accepted 2 October 2019
Available online 23 October 2019

Keywords:
Alginate
Fruit juice
Fruit
Lactobacillus casei
Low pH
Microencapsulation
Pasteurization
Probiotics
Vibration technology
Anthocyanins

A B S T R A C T

Background: Foods including probiotics are considered "functional foods." As an alternative to dairy products, we investigated the behavior of *Lactobacillus casei* when exposed to low-pH fruit juice. Juices of fruits such as pineapple, raspberry, and orange were assessed. Free and microencapsulated forms of *L. casei* were compared, and the viability of the probiotic was evaluated under storage at 4°C for 28 d. Microbiological analyses were carried out to ensure a safe and healthy product for consumers who look for foods with probiotics from sources other than dairy.

Results: Low pH affected *L. casei* survival during storage depending on the type of fruit juice. In the case of pineapple juice, some microcapsules were broken, but microcapsules recovered at the end of the storage period had 100% viability (2.3×10^7 CFU/g spheres). In the case of orange juice, more than 91% viability (5.5×10^6 CFU/g spheres) was found. In raspberry juice, viability decreased rapidly, disappearing at the end of the storage period, which was caused by the absorption of high concentrations of anthocyanin inside microcapsules more than low pH.

Conclusion: Low pH affected the survival of *L. casei* under refrigeration; even when they were microencapsulated, acidic conditions impacted their viability. Although pH affects viability, its value is very sensitive and will depend on the type of fruit juice and its composition. Some fruit juices contain compounds used as substrates for *Lactobacillus* and other compounds with antimicrobial effects.

How to cite: Olivares A, Soto C, Caballero E, et al. Survival of microencapsulated *Lactobacillus casei* (prepared by vibration technology) in fruit juice during cold storage. Electron J Biotechnol 2019;42. https://doi.org/10.1016/j.ejbt.2019.10.002.

1. Introduction

Probiotics are increasingly used as food supplements because of the recognized scientific evidence supporting the concept that maintenance of healthy gut microflora may provide protection against gastrointestinal disorders including infections and inflammatory syndromes of the bowel, relief of constipation, and prevention of urinary tract infection as well as immunostimulatory effects [1,2,3,4]. The FAO/WHO definition of a probiotic is "live microorganisms that when administered in adequate amounts confer a health benefit on the host" [5]. To exert their health benefits, the minimum concentration of probiotics in food products is suggested to be one that results in a level of live probiotic cells of approximately 10^6–10^7 CFU/mL or CFU/g at the end of the shelf life of a product [6].

Traditionally, commercial products containing probiotics have been related to dairy products such as yogurt, ice cream, and cheese. Nondairy probiotic products are a new alternative for consumers who do not enjoy or cannot consume dairy products, including people who are lactose intolerant, allergic to milk proteins, hypercholesterolemic, or strictly vegetarian [7].

Fruit juices are suitable products for supplementation with *Lactobacillus* as a probiotic.

Fruit juices are consumed by people of all ages and are considered healthy and refreshing beverages. In addition, fruit juices do not contain starter cultures, which compete for nutrients with probiotic cultures [6,8,9].

The pH of fruit juices is typically low, between pH 2.5 and 3.7; however, probiotic bacteria are extremely sensitive to acidic conditions. Several studies have been carried out to investigate the behavior of free probiotics in fruit juice during refrigeration storage [7,8,9,10,11,12,13,14]. Microencapsulation techniques have received considerable attention and may be a suitable carrier system to enhance the ability of probiotics to survive under extreme conditions such as low pH [4,15,16,17].

* Corresponding author.
E-mail address: araceliolivares@creas.cl (A. Olivares).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2019.10.002

Depending on the method used to form the beads, the encapsulation technique can be classified into two groups: extrusion (droplet method) and emulsion or two-phase system, where extrusion is evolved in the vibration technology that is based on the principle that a laminar-flowing liquid jet breaks up into equal-sized droplets by a superimposed vibration [4].

Sodium alginate is the most popular encapsulation matrix because of its simplicity, nontoxicity, low cost, biocompatibility, and acceptability as a food additive [4,18]. Alginates occur both as structural components in marine brown algae (Phaeophyceae) and as capsular polysaccharides in some bacteria. Alginates are binary, linear copolymers of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues of widely different composition and sequence [19].

Further, limited knowledge exists relating to microencapsulated probiotics and their survival in fruit juice during storage [3,6,11,20,21].

The aim of this study was to evaluate the behavior of *Lactobacillus casei* (DSM 20011) when exposed to low-pH fruit juices. This probiotic strain was previously shown to have acidic resistance in simulated gastric juice [4]. Free and microencapsulated forms were compared, and the viability of the probiotic was evaluated under storage conditions at 4°C for 28 d. Natural (no preservatives) and pure (no water) pineapple juice (pH 3.28), raspberry juice (pH 2.75), and orange juice (pH 3.45) were assessed.

Microencapsulation by the vibration technique used here was developed with a consistent and sophisticated encapsulation technology provided by BÜCHI, which allowed reproducibility, narrow size distribution of the spheres obtained, and a better method of comparison, for example, based on the microencapsulation yield and viability of strain [4].

2. Materials and methods

2.1. Growth and microencapsulation conditions of probiotic bacteria

L. casei (DSM 20011) was supplied by DSMZ-Germany and was stored in 2% glycerol at −80°C. The strain was grown in Man, Rogosa, and Sharpe (MRS) broth (Difco, BD USA) according to the method given by Olivares et al. [4]. Finally, a lyophilized powder with more than 10^{10} CFU/g was obtained.

A sophisticated microencapsulation technique (using vibration technology) developed by BÜCHI, Germany [model Encapsulator B-390] (CIENTEC Instrumentos Científicos S.A, Chile) was used according to the procedure described by Olivares et al. [4]. An aqueous suspension of lyophilized *L. casei* (mentioned above with 10^{10} CFU/g) at 5 g/L was prepared and mixed (1:1) with 2% sodium alginate solution. Microcapsules were prepared using 1000 Hz, 20 mL/min, and a 450 μm nozzle size (Encapsulator parameters), and the size of the microcapsules was approximately between 500 μm and 700 μm [4]. Encapsulation yield (EY), which is a combined measurement of the efficacy of entrapment and the survival of viable cells, was calculated using [Equation 1] [4,15]:

$$EY = (N/No) \times 100 \tag{1}$$

where N is the number of viable entrapped cells released from the microcapsules, and No is the number of free cells added to the biopolymer mixture during the production of the microcapsules [4].

The viability of the microencapsulated *L. casei* used in subsequent trials was 7.362 log CFU/g spheres.

2.2. Fruit juice preparation and pasteurization process

Three favorite consumer fruits (also consumed as juice) were selected on the basis of their low pH values. Oranges and pineapples, obtained from a local market (Mercado Cardonal, Valparaíso, Chile), and frozen, locally sourced raspberries were used.

To obtain fruit juices (after washing, peeling, and/or cutting fruits), a juice extractor or a citrus juicer was used for pineapples and raspberries or oranges, respectively. Juices were not filtered. They were used directly from the juice extractor and citrus juicer containers. No additional compounds such as preservatives or water were added. Pineapple, raspberry, and orange juice pH values were 3.28, 2.75, and 3.45, respectively.

All juices were subjected to thermal pasteurization following a procedure adapted from Polydera et al. [22] and Sheehan et al. [9]. Forty milliliters of juice was taken in a 100-mL sealed Schott flask, and the flask was incubated in a thermal circulation water bath [WiseCircu, *Dalthan Scientific* Co., Ltd., Korea]. One flask with a thermometer inside was used as a control and to count time.

After that, all juices were stored immediately at 4°C in a home refrigerator. The pH was not adjusted.

The pasteurized juices were incubated on an MRS agar plate to verify whether *Lactobacillus* growth from the juice and did not contaminate the *Lactobacillus* added in the assays. Aerobic count (AC) and yeast and molds (YM) were tested using a Petrifilm™ plate by 3 M™ [manufacturer's instructions were followed].

2.3. Microencapsulated and free Lactobacillus added to juices

To study acid tolerance in the juices, 4.0 g of microencapsulated *L. casei* (7.362 log CFU/g spheres) and 2 mL of a solution with free *L. casei* were added to 40 mL of each pasteurized juice (pineapple, orange, and raspberry) and maintained under refrigeration at 4°C. Samples were taken (after the microcapsules had settled) at 0, 7, 14, 21, and 28 d, and colonies were counted and expressed as CFU (or $\log_{10}$ CFU) per mL of juice. Once the trials were concluded, at 28 d, the microcapsules were recovered by filtration (Whatman® Grade 1 filter paper, provided by Sigma-Aldrich), and then the microcapsules were rinsed three times with distilled water. CFU were counted as described below.

2.4. Microencapsulated cell count

The microcapsules were dissolved in 50-mM sodium citrate (pH 7.5) to release *Lactobacillus* cells into the supernatant for counting. Appropriate dilutions of the supernatant were then plated on MRS agar (Difco, BD, USA) and aerobically incubated for 48–72 h at 37°C (Incubator Labtech LIB-150M; Daihan Labtech Co. Ltd., Korea). Then, the microencapsulated *L. casei* were enumerated as CFU (or $\log_{10}$ of CFU) per gram of spheres [4].

2.5. Statistical analysis

The results are expressed as the mean ± standard deviation of triplicate experiments. Single-factor ANOVA was used to compare results and determine statistically significant difference ($P < 0.05$).

3. Results and discussion

3.1. Pasteurization process

To carry out the pasteurization process, two different conditions were assayed: condition 1 (63°C and 30 min) and condition 2 (72°C and 90 s), adapted from Polydera et al. [22] and Sheehan et al. [9]. Fig. 1 shows CFU/mL growth in MRS agar (MRS) on a Petrifilm plate for AC and for YM under pasteurization conditions 1 (C1) and 2 (C2) for pineapple juice (other juices not shown). Mo represents nonpasteurized pineapple juice. The results show that some colonies grew in the MRS agar, a specific medium for lactic acid bacteria, even when the juice was nonpasteurized (Mo), to 2.96×10^4 CFU/mL at

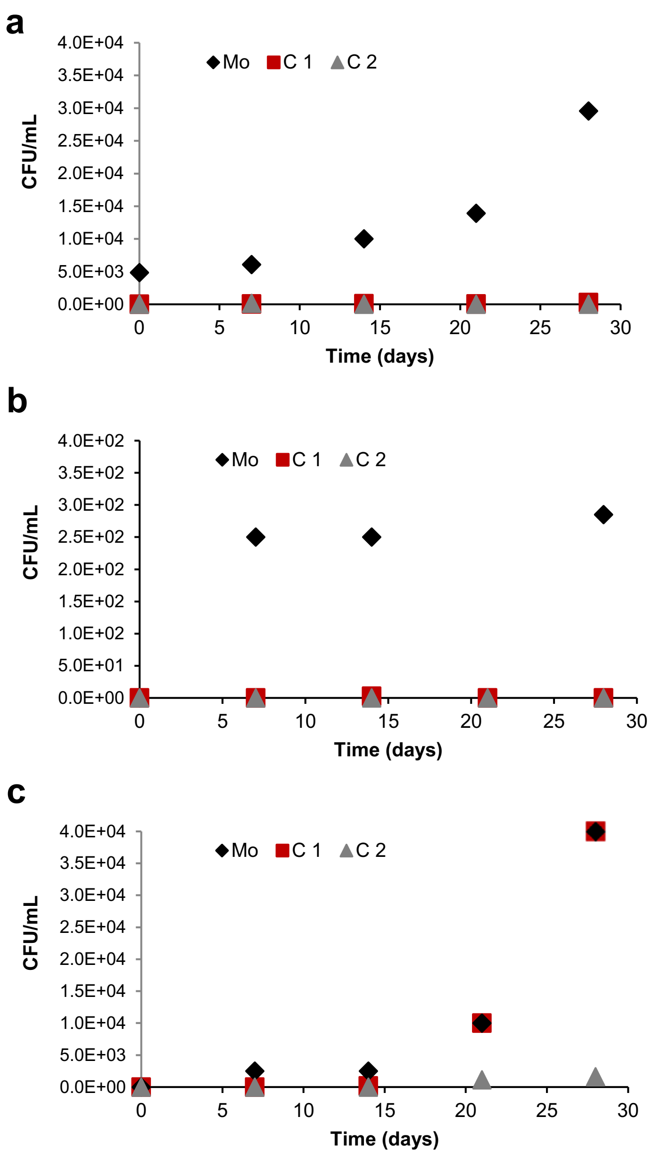

Fig. 1. Colony count in different plates under the two pasteurization conditions of pineapple juice. (a): MRS agar (specific for *Lactobacillus*); (b): AC (aerobic count); (c): YM (yeast and mold). Mo: unpasteurized; C1: condition 1 (63°C and 30 min); C2: condition 2 (72°C and 90 s).

28 d. Similar colonies were observed in raspberry and orange juices and appeared after 2 weeks, totaling to less than 10^3 CFU/mL. In these cases, the colonies were light pink (not white-yellow like *Lactobacillus*), and the absence of *Lactobacillus* was determined by microscopy (Olympus U-*CTR* 30-2, Tokyo, Japan) at 100× oil magnification.

MRS is a selective culture medium for lactic acid bacteria. In addition to *Lactobacillus*, and other genera such as *Enterococcus, Weissella,* and *Leuconostoc,* commonly found as endogenous microbiota in fruits, can be routinely isolated and counted in this medium [23].

After the pasteurization process, for C1, the number of light pink colonies was 2.60×10^2 CFU/mL at 28 d, and for C2, the undesirable colonies grown in the MRS agar were completely removed.

For AC, Fig. 1a shows a similar tendency as that in the MRS plate in the case of unpasteurized juice (note the different scale in CFU/mL), reaching 2.85×10^2 CFU/mL at 28 d, while the presence of colonies was zero in pasteurized juice under C1 and C2.

The Petrifilm YM count plate (Fig. 1c) uses a phosphatase indicator dye. In the case of pineapple juice, this fruit contains acid phosphatase that reacts with the gel included in the plate and generates a blue color. This coloration alters the count plate in the unpasteurized sample (Mo); however, yeast and molds appear clearly after 1 week and then increase. For C1 and C2, the blue coloration disappeared, and acid phosphatase was inactivated at temperatures of more than 60°C; therefore, no reaction occurred with the count plate gel. In this case, at 14 d, some colonies in C1 (2.05×10^2 CFU/mL) reached more than 4.0×10^4 CFU/mL at 28 d. Additionally, some colonies (YM) were detected in C2, although they were lower than 1.65×10^3 CFU/mL at 28 d. It is likely that some spores could not be destroyed by pasteurized conditions. Finally, condition 2 (C2) was selected for future trials.

3.2. Behavior of Lactobacillus during storage in fruit juices

The pH values of the fruit juices used in this study were between 2.75 and 3.45. Microencapsulation can provide a good opportunity for *L. casei* to resist acidic conditions (an earlier study was conducted, and this strain was selected for its resistance under acidic conditions, such as in simulated gastric juice [4]).

Fig. 2 represents the viability of *L. casei* in pineapple juice stored at 4°C for 28 d in the microencapsulated and free forms. Microencapsulated probiotics increased the count in the juices, which means that some microcapsules did not resist acidity of the juice, and *Lactobacillus* were released from the microcapsules into the juice. After 28 d, more than 65% of microcapsules recovered with 2.3 × 10^7 CFU/g spheres, which was the same value as that of the initial microcapsules added to the pineapple juice. Thus, the microcapsules that remained intact in the juice did not decrease their amount of *Lactobacillus*, which confirms that sodium alginate can protect them. However, at low pH, the integrity of the microcapsules was affected, allowing the release of *Lactobacillus* from them and to maintain viability in juice as well as free *Lactobacillus*.

In the case of free *Lactobacillus* in the pineapple juice, the count remained almost constant during the storage period (greater than 95% viability). The viability of free *Lactobacillus* in the pineapple juice (pH 3.28) was limited by resistance to acidic conditions. Olivares et al. [4] reported that in simulated gastric juice, *L. casei* did not survive after 2 h at pH 3.0, while at pH 3.5, more than 95% survival was obtained. However, pineapple juice was most likely used as a substrate (because of the high sugar content) to maintain almost the total probiotic concentration at the end of the storage period. The pH of the pineapple juice was 3.28; however, the viability of the free *Lactobacillus* and the microencapsulated *Lactobacillus* in the juice (released from the microcapsules) was high at the end of the storage period for both forms, which means that (independent of the pH effect) some *Lactobacillus* could grow in the juice using the juice carbohydrates (mainly sucrose). A similar study was carried out by Costa et al. [8] evaluating the use of sonicated pineapple juice as a substrate for producing a probiotic beverage by *L. casei* NRRL B442 (nonmicroencapsulated). The genus *Lactobacillus* can grow in the pH range from mild acid to neutral values and at temperatures from 2 to 53°C, with optimum temperature range of 30–40°C and optimum pH range of 5.5–6.2; however, pH decreases during storage (temperature of 4°C) due to lactic acid production when the *Lactobacillus* use sugar present in the juices as a substrate [8].

Sheehan et al. [9] showed similar results with *L. casei* (DN-114001) storage for 12 weeks at 4°C, with greater than 77% survival at the end of storage period and greater than 98% for a 4-week storage period.

Fig. 3 shows the viability of *L. casei* in orange juice stored at 4°C for 4 weeks in the microencapsulated and free forms.

Microencapsulated probiotics increased the count in orange juice after the first week, and the *Lactobacillus* count was 7.0×10^4 CFU/mL after 28 d; 59.3% of microcapsules were recovered with 5.5×10^6 CFU/g spheres, corresponding to a survival of 91%, which is the same value as that of the initial microcapsules added to the orange juice (pH 3.45).

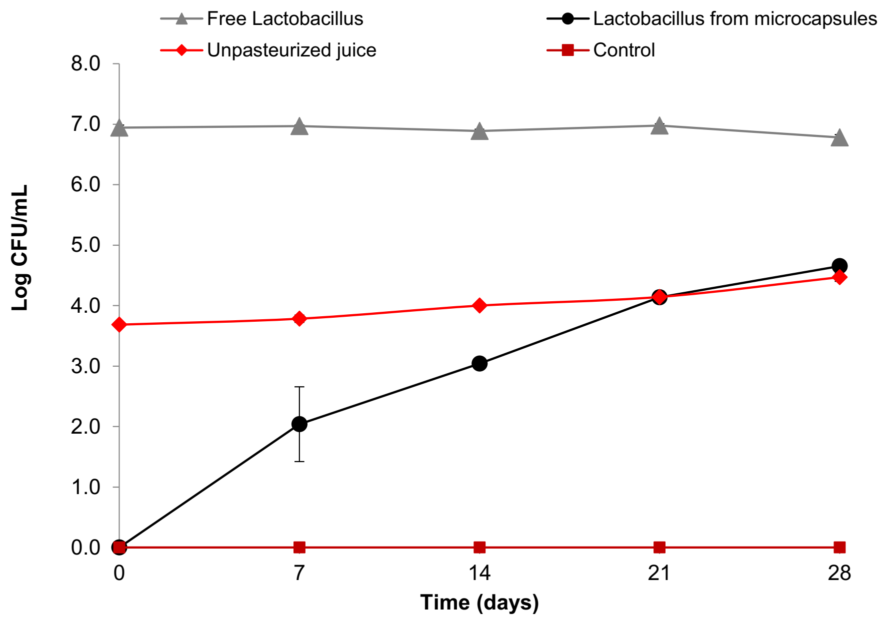

Fig. 2. Viability of the microencapsulated and free *Lactobacillus casei* in pineapple juice stored at 4°C for 28 d. The error bars represent the standard deviation (n = 3).

In the case of free *Lactobacillus* in the orange juice, the count decreased after 14 d of storage at 4°C, and at 21 d, the free *Lactobacillus* almost disappeared; however, at the end of the storage period, the count was 1.0×10^3 CFU/mL. The effect of Vitamin C (as an antimicrobial compound) was stronger than the capacity to grow using the carbohydrates present in the orange juice, but it can also be used as a substrate [23]. Da Costa et al. [24] used prebiotic or ascorbic acid supplementation on *Lactobacillus paracasei ssp. paracasei* cultures in orange juice during cold storage (4°C for 28 d); however, these compounds did not exert a protective effect on probiotic viability during storage, and the juices showed viability higher than 10^6 CFU/mL during 28 d of cold storage, showing that pure orange juice is a suitable substrate for *L. paracasei* propagation. On the other hand, ascorbic acid or vitamin C is an important compound present in orange juice, with known antioxidant and antimicrobial properties [25]. However, high concentrations of citric acid and vitamin C present in orange juice are important nutrients and can be metabolized by *L. casei* and used as energy sources [3]. Miranda et al. [3] compared the direct (direct addition of the commercial probiotic culture), activated (addition of probiotic culture activated by propagation), and encapsulated (addition of the encapsulated probiotic culture) additions in orange juice and concluded that the probiotic addition methodology had no significant influence on probiotic culture survival. In general, it is difficult to maintain the viability of probiotic cultures in fruit juices because these products have a low pH and a high concentration of dissolved oxygen and insufficient amounts of free amino acids and peptides. The viability of *Lactobacillus* in fruit juices will depend on the physical and chemical parameters of the

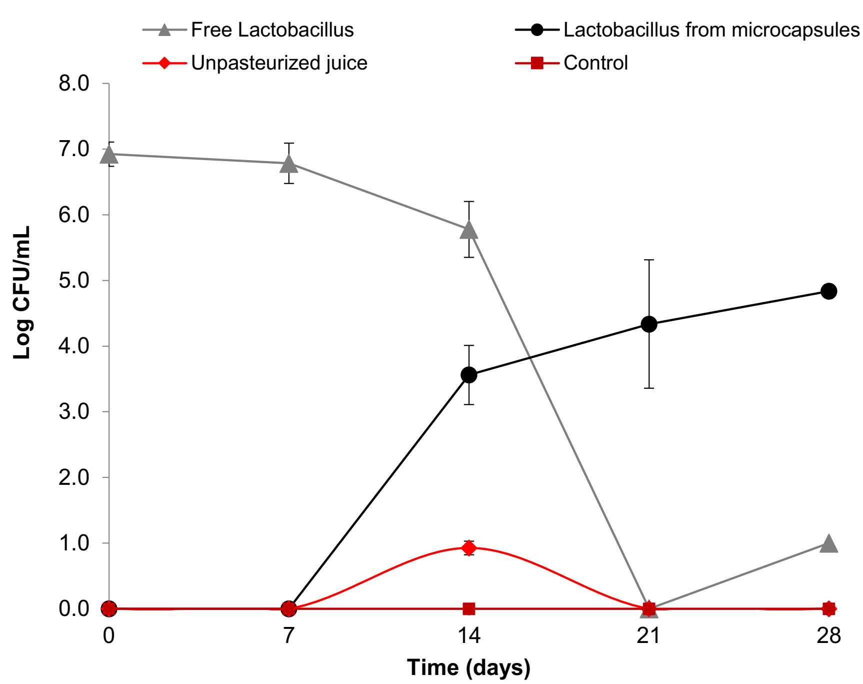

Fig. 3. Viability of the microencapsulated and free *Lactobacillus casei* in orange juice stored at 4°C for 28 d. The error bars represent the standard deviation (n = 3).

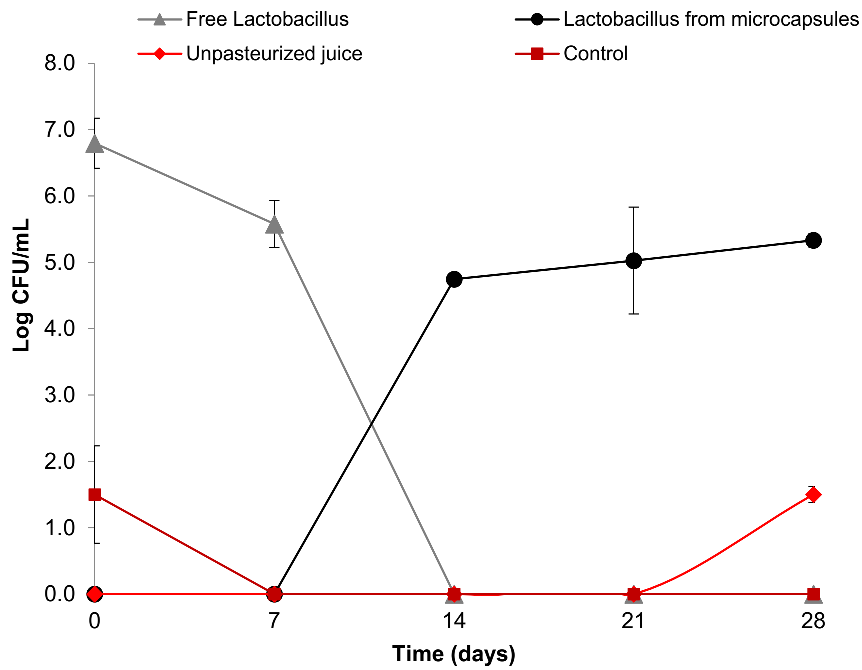

Fig. 4. Viability of microencapsulated and free *Lactobacillus casei* in raspberry juice stored at 4°C for 28 d. The error bars represent the standard deviation (n = 3).

juice and the probiotic strain used. The strains used in food are usually aerobic or microaerophilic; therefore, the presence of oxygen in the product can cause toxicity and loss of viability. For its part, Vitamin C could have a protective effect on lactobacilli during storage, probably because it is an oxygen scavenger, thus promoting a more favorable anaerobic environment, but this does not occur at the beginning [24].

Fig. 4 shows the viability of *L. casei* in raspberry juice stored at 4°C for 4 weeks in the microencapsulated and free forms. Microencapsulated *L. casei* in raspberry juice (pH 2.75) showed a different behavior than that of pineapple and orange juices in terms of viability.

Microencapsulated probiotics in raspberry juice are released in the medium at day 7 and increase slightly more toward the end of the storage period. After that, 47.6% of microcapsules were recovered by filtration; however, the viability of these microcapsules was null.

Table 1
Summary of probiotic viability in microcapsules and in juice (with free and microencapsulated *Lactobacillus*) at 28 d of refrigeration storage (4°C).

Fruit juice	Juice pH[a]	Recovered microcapsules	Viability of recovered microcapsules[b]	Viability in juice with microcapsules[c]	Viability in juice with free *Lactobacillus*[d]
Pineapple 	3.28	65.1%	100%	>4.0×10^4	>84.5%
Raspberry 	2.75	47.6%	ND	>2.2×10^5	ND
Orange 	3.45	59.3%	> 91%	>7.0×10^4	<14%

ND: non detected.
[a] Natural pH of fruit juice prepared in the laboratory with detailed information of equipment in the methodology section.
[b] Percentage (%) of viability of *Lactobacillus casei* in the recovered microcapsules with regard to the initial viability of the added microcapsules.
[c] *Lactobacillus* count that are released from the microcapsules to the juice (CFU/mL).
[d] Percentage (%) of viability of free *Lactobacillus casei* that remained in juice with regard to the initial viability of the added free *Lactobacillus*.

Microcapsules recovered from raspberry juice acquired a dark pigmentation associated with anthocyanins present in raspberry juice. This pigmentation of microcapsules is attributed to the loss of viability because anthocyanins, within their multiple properties (mainly as an antioxidant), also have antibacterial properties. In this sense, it is believed that the sodium alginate absorbed these compounds, eliminating the *Lactobacillus* present in microcapsules because in the fruit juice; the count of *Lactobacillus* was higher than that obtained in other juices, not being affected by anthocyanins, but by the pH. The survival of microencapsulated single- and double-chitosan-coated alginate *Lactobacillus plantarum* was studied in pomegranate and cranberry juices, which also contain high amounts of phenolic compounds, and it was shown that the coating matrix was able to inhibit the penetration of phenolic compounds within the beads [26], which is another probable mechanism through which microcapsules could protect *Lactobacillus* from the antimicrobial activity of compounds present in raspberry juice, such as anthocyanins.

For free *L. casei*, a dramatic loss of viability occurred at 7 d, and a total absence occurred at 14 d. Among the three juices studied, raspberry was found to have the lowest pH (2.75). This strain was previously studied in the simulated gastric medium [4], and at pH less than 2.5, it survived only for 60 min, while at pH 3.0, the viability was zero at 120 min.

Cornelian cherry juice [10], known as a good source of anthocyanin and phenolic compounds and with a pH of 2.6, as low as that of raspberry juice, was found to be very detrimental to strains (native and industrial *L. casei*), and probiotics were completely diminished after approximately 7 d of refrigerated storage. However, following a pH-adjusted treatment (to pH 3.5), the viability of the strain was improved to 28 d [10].

Nualkaekul et al. [11] studied the survival of *L. plantarum* NCIMB 8826 in model solutions and developed a mathematical model describing its dependence on pH. The model predicted *Lactobacillus* survival well in orange, blackcurrant, and pineapple juices; however, it failed to predict cell survival in grapefruit and pomegranate juices, indicating the influence of other factors (such as citric acid and ascorbic acid), in addition to pH, on the viability of free *Lactobacillus*.

A summary of *L. casei* viability inside of recovered microcapsules and in medium juice (*Lactobacillus* released from microcapsules) at the end of the storage period is presented in Table 1.

Table 2
Microbiological analyses (count in plate, CFU/mL) to fruit juices at 28 d of refrigeration storage (4°C). AC: aerobic count; YM: yeast and mold; m: juice with microencapsulated *Lactobacillus*; f: juice with free *Lactobacillus*.

Fruit juice	AC m	AC f	YM m	YM f	*E. coli* m
Pineapple 	<100	<660	<40,000	<120,000	Negative
Raspberry 	<53	Negative	<3000	<100	Negative
Orange 	<103	Negative	<1140	Negative	Negative

In the case of free *Lactobacillus*, it is possible to observe that the viability depends not only on fruit juice pH but also on other factors. Because pineapple juice has a lower pH than orange juice, the viability of free *Lactobacillus* was higher. Different compounds present in fruits affect viability in different ways, such as by affecting the carbon source, phenolic compounds (such as anthocyanins in raspberries), or vitamin C (in oranges).

In the case of pineapple and orange juices, the amount of *L. casei* in microcapsules and that released into the juice reach the recommended level of viable probiotics to achieve health benefits (greater than 10^6 CFU/mL) at the end of the refrigerated storage period (28 d). In the case of raspberry juice, external factors (such as anthocyanins) did not allow the microencapsulated *L. casei* to survive; however, some probiotics released in the juice survived (more than 10^5 CFU/mL).

Additionally, to establish the safety of the juices evaluated at the end of the refrigerated storage period (28 d), some microbiological analyses were performed. As in the case of the pasteurization process, AC and YM were tested using a Petrifilm plate by 3 M, including *E. coli* control with the Petrifilm plate by 3 M. This last assay was negative for the 3 juices. For AC, the Chilean sanitary regulation of food (based in Codex Alimentarius) demands that pasteurized fruit juice have from 10^2 to 10^3 CFU/mL, making the fruit juice a safe and healthy beverage (Table 2).

4. Conclusion

According to this study, low pH affected the survival of *L. casei* under refrigeration; even when *L. casei* were microencapsulated, acidic conditions impacted their viability [4]. Although pH affects viability, its value is very sensitive and will depend on the fruit juice type and its composition (sugar content, vitamins, enzymes, and phenolic compounds, among others). Some fruit juices contain compounds used as substrates for *Lactobacillus* and other compounds with antimicrobial effects.

Among the fruit juices tested, microencapsulated probiotics survived well in pineapple and orange juices, whereas in raspberry juice, survival decreased rapidly and disappeared at the end of the storage period because of the absorption of high levels of anthocyanins inside of the microcapsules rather than by low pH. However, the sum of the amount of the microencapsulated form and the free form (released from the microcapsules) of *L. casei* makes fruit juices an alternative, nondairy food matrix for supplementation with probiotic bacteria.

It can be deduced that in certain juices, some compounds seemed to protect the probiotics during storage; in contrast, in certain juices such as raspberry, *Lactobacillus* viability was lower, which could be due to the presence of antimicrobial compounds such as anthocyanins.

On the other hand, all information will provide and open new doors to improve the microencapsulation process and allow future research to include probiotic organisms in nondairy foods, hence expanding the supply of these functional foods.

This work could contribute new information for practical proposes of easy replicate, mainly because the strain, matrix, fruits, and equipment used to prepare microcapsules are commercially available (vibration technology).

Conflict of interest

None.

Acknowledgments

Financial support by FONDECYT Iniciación No 11130460 (CONICYT - Chile); and Centro Regional de Estudios en Alimentos Saludables (CREAS), Regional Program, Chile, GORE-CONICYT R17A10001.

References

[1] Cáceres P, Gotteland M. Alimentos probióticos en Chile: ¿Qué cepas y qué propiedades saludables? Rev Chil Nutr 2010;37(1):97–109. https://doi.org/10.4067/S0717-75182010000100010.
[2] De Seta F, Parazzini F, De Leo R, et al. *Lactobacillus plantarum* P17630 for preventing Candida vaginitis recurrence: a retrospective comparative study. Eur J Obstet Gynecol Reprod Biol 2014;182:136–9. https://doi.org/10.1016/j.ejogrb.2014.09.018 PMid: 25305660.
[3] Miranda R, De Paula M, Da Costa G, et al. Orange juice added with *L. casei*: is there an impact of the probiotic addition methodology on the quality parameters? LWT-Food Sci Technol 2019;106:186–93. https://doi.org/10.1016/j.lwt.2019.02.047.
[4] Olivares A, Silva P, Altamirano C. Microencapsulation of probiotics by efficient vibration technology. J Microencapsul 2017;34(7):667-64. https://doi.org/10.1080/02652048.2017.1390005 PMid: 28985684.
[5] Hill C, Guarner F, Reid G, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506–14. https://doi.org/10.1038/nrgastro.2014.66 PMid: 24912386.
[6] Ding WK, Shah NP. Survival of free and microencapsulated probiotic bacteria in orange and apple juices. Int Food Res J 2008;15(2):219–32.
[7] Bevilacqua A, Campaniello D, Corbo MR, et al. Suitability of *Bifidobacterium* spp. and *Lactobacillus plantarum* as probiotics intended for fruit juices containing citrus extracts. J Food Sci 2013;78(11):M1764–71. https://doi.org/10.1111/1750-3841.12280 PMid: 24245895.
[8] Costa MGM, Fonteles TV, de Jesus ALT, et al. Sonicated pineapple juice as substrate for *L. casei* cultivation for probiotic beverage development: process optimisation and product stability. Food Chem 2013;139(1–4):261–6. https://doi.org/10.1016/j.foodchem.2013.01.059 PMid: 23561104.
[9] Sheehan V, Ross P, Fitzgerald G. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. Innov Food Sci Emerg Technol 2007;8(2):279–84. https://doi.org/10.1016/j.ifset.2007.01.007.
[10] Nematollahi A, Sohrabvandi S, Mortazavian AM, et al. Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. Electron J Biotechnol 2016;21:49–53. https://doi.org/10.1016/j.ejbt.2016.03.001.
[11] Nualkaekul S, Charalampopoulos D. Survival of *Lactobacillus plantarum* in model solutions and fruit juices. Int J Food Microb 2011;146(2):111–7. https://doi.org/10.1016/j.ijfoodmicro.2011.01.040 PMid: 21411170.
[12] Champagne CP, Raymond Y, Gagnon R. Viability of *Lactobacillus rhamnosus* R0011 in an apple-based fruit juice under simulated storage conditions at the consumer level. J Food Sci 2008;73(5):M221–6. https://doi.org/10.1111/j.1750-3841.2008.00775.x PMid: 18577004.
[13] Shori AB. Influence of food matrix on the viability of probiotic bacteria: a review based on dairy and non-dairy beverages. Food Biosci 2016;13:1–8. https://doi.org/10.1016/j.fbio.2015.11.001.
[14] Srisukchayakul P, Charalampopoulos D, Karatzas KA. Study on the effect of citric acid adaptation toward the subsequent survival of *Lactobacillus plantarum* NCIMB 8826 in low pH fruit juices during refrigerated storage. Food Res Int 2018;111:198–204. https://doi.org/10.1016/j.foodres.2018.05.018 PMid: 30007676.
[15] Chávarri M, Marañón I, Villagrán MC. Encapsulation technology to protect probiotic bacteria. In: Cis E, editor. Probiotics, vol. 23; 2012. p. 501–40. https://doi.org/10.5772/50046.
[16] De Prisco A, Maresca D, Ongeng D, et al. Microencapsulation by vibrating technology of the probiotic strain *Lactobacillus reuteri* DSM 17938 to enhance its survival in foods and in gastrointestinal environment. LWT-Food Sci Technol 2015;61(2):452–62. https://doi.org/10.1016/j.lwt.2014.12.011.
[17] Martín MJ, Lara-Villoslada F, Ruiz MA, et al. Microencapsulation of bacteria: a review of different technologies and their impact on the probiotic effects. Innov Food Sci Emerg Technol 2015;27:15–25. https://doi.org/10.1016/j.ifset.2014.09.010.

[18] Chaikham P. Stability of probiotics encapsulated with Thai herbal extracts in fruit juices and yoghurt during refrigerated storage. Food Biosci 2015;12:61–6. https://doi.org/10.1016/j.fbio.2015.07.006.
[19] Draget K, Taylor C. Chemical, physical and biological properties of alginates and their biomedical implications. Food Hydrocoll 2011;25(2):251–6. https://doi.org/10.1016/j.foodhyd.2009.10.007.
[20] Doherty SB, Auty MA, Stanton C, et al. Application of whey protein micro-bead coatings for enhanced strength and probiotic protection during fruit juice storage and gastric incubation. J Microencapsul 2012;29(8):713–28. https://doi.org/10.3109/02652048.2011.638994 PMid: 22970750.
[21] Polydera AC, Storoforos NG, Taoukis PS. Comparative shelf-life study and vitamin C loss kinetics in pasteurized and high pressure processed reconstituted orange juice. J Food Eng 2003;60(1):21–9. https://doi.org/10.1016/S0260-8774(03)00006-2.
[22] Trias R, Bañeras L, Montesinos E, et al. Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. Int Microbiol 2008;11(4):231–6. https://doi.org/10.2436/20.1501.01.66 PMid: 19204894.
[23] Zoghi A, Khosravi-Darani K, Sohrabvandi S, et al. Production of synbiotic apple juice and evaluation of viable count of Lactobacillus Plantarum ATCC 8014 during refrigerated storage. Biointer Res Appl Chem 2018;8(6):3674–81.
[24] Da Costa GM, Silva JVC, Mingotti JD, et al. Effect of ascorbic acid or oligofructose supplementation on *L. paracasei* viability, physicochemical characteristics and acceptance of probiotic orange juice. LWT- Food Sci Technol 2017;75:195–201. https://doi.org/10.1016/j.lwt.2016.08.051.
[25] Kallio J, Jaakola M, Mäki M, et al. Vitamin C inhibits *Staphylococcus aureus* growth and enhances the inhibitory effect of quercetin on growth of *Escherichia coli in vitro*. Planta Med 2012;78(17):1824–30. https://doi.org/10.1055/s-0032-1315388 PMid: 23059632.
[26] Nualkaekul S, Cook MT, Khutoryanskiy VV, et al. Influence of encapsulation and coating materials on the survival of Lactobacillus plantarum and Bifidobacterium longum in fruit juices. Food Res Int 2013;53:304–11. https://doi.org/10.1016/j.foodres.2013.04.019.