Evaluation of yogurt and various beverages as carriers of lactic acid bacteria producing 2-branched (1,3)-β-D-glucan

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ABSTRACT

Probiotic cultures are increasingly being incorporated into a wide variety of food products. Although lactobacilli and bifidobacteria are the most frequently used, other lactic acid bacteria (LAB) have been reported to be potential probiotics. Of these, the cider isolates *Pediococccus parvulus* (strains 2.6 and CUPV22) and *Lactobacillus suebicus* CUPV221 produce a 2-branched (1,3)-β-D-glucan exopolysaccharide that decreases serum cholesterol levels and affects the activation of human macrophages. For this reason, these 3 strains were incorporated into yogurt, orange juice, and 2 juice-milk beverages to evaluate the effect of the food matrix on the resistance of these strains to simulated gastrointestinal tract conditions. Our results showed that incorporation of the LAB did not significantly affect the physical and rheological properties of the food matrices tested. When incorporated in yogurt, LAB strains population decreased by 2 to 3 log orders of magnitude during the shelf life of the product (28 d). However, no significant decrease was observed in the juice and juice-milk beverages during the same storage period, except for *Lb. suebicus*, whose viability decreased by 3 log orders of magnitude. When strains were subjected to gastrointestinal tract conditions, a decrease in the survival was observed at the lower pH (1.8). However, incorporation of these LAB strains into orange juice increases their resistance to lower pH conditions, thus improving survival to gastrointestinal stress. Moreover, a protective effect was observed for *P. parvulus* CUPV22 and 2.6 to gastric stress in juice-milk beverages and to gastrointestinal stress in yogurt. *Lactobacillus suebicus* CUPV221 did not survive when incorporated into yogurt and juice-milk beverage.

INTRODUCTION

Probiotics are the most widely used microorganisms in the manufacture of functional foods and are defined as "living microorganisms which, when administered in adequate amounts, confer health benefits on the host" (FAO/WHO 2002). In this sense, increasing evidence suggests that the consumption of lactic acid bacteria (**LAB**) may improve lactose assimilation and food digestibility, or may prevent hypercholesterolemia, intestinal infections, cancer, or food allergies (Richardson, 1996; Kailasapathy and Rybka, 1997; Mattila-Sandholm et al., 1999; Shah, 2000). The most widely used probiotic bacteria are lactobacilli and bifidobacteria, mostly isolated from the human gastrointestinal tract (Champagne, 2009). However, strains of LAB from other genera or species such as *Enterococcus faecalis, Enterococcus faecium, Lactococcus lactis, Leuconostoc mesenteroides*, and *Pediococcus acidilactici* have been reported to be potential probiotics (Holzapfel et al., 2001; de Vrese and Schrezenmeir, 2008). Moreover, identification and characterization of probiotic strains from diverse habitats such as cider or wine may extend the number of probiotic microorganisms for production of functional foods. Recent studies have demonstrated that 2-branched (1,3)-β-D-glucan–producing strains isolated from cider exhibit probiotic properties (Foligné et al., 2010). Among these, *Pediococcus parvulus* 2.6 is resistant to gastrointestinal stress (Immerstrand et al., 2010), adheres to Caco-2 cells, and induces the production of inflammation-related cytokines by polarized macrophages (Fernández de Palencia et al., 2009). Furthermore, *Pediococcus parvulus* CUPV22 and CUPV1 strains, as well as *Lactobacillus suebicus* CUPV221, are resistant to pancreatin and bile salt, adhere to intestinal epithelial human cells, and could activate human macrophages (Garai-Ibabe et al., 2010b). In addition,

Lb. suebicus stimulated the production of the cytokine IL-10 (Garai-Ibabe et al., 2010b). Therefore, these 2-branched (1,3)-β-D-glucan–producing bacteria have potential for elaboration of functional foods and were used in the current study. In addition, human trials of an oat-based food elaborated with *P. parvulus* 2.6 resulted in a decrease in serum cholesterol levels and increased counts of fecal *Bifidobacterium* spp. (Mårtensson et al., 2005). It has also been reported that an oat fiber concentrate fermented with *P. parvulus* 2.6 provoked changes in short-chain fatty acid formation in the cecum, distal colon, and feces of rats, which may be beneficial to colonic health (Lambo-Fodje et al., 2006).

Many food products are used as probiotic carriers, with dairy products being the most commercially important (Champagne, 2009). Due to their perceived health properties, yogurt and fermented milks have been used as probiotic carriers for the past 2 decades (Mattila-Sandholm, 2002). Probiotic yogurts may contain, besides the traditional starter cultures *Lactobacillus delbrueckii* and *Streptococcus thermophilus*, strains that have been shown to produce beneficial effects in the human gut, mainly *Lb. acidophilus* and *Bifidobacterium* spp. (Champagne et al., 2005). Although the addition of microorganisms to other food matrices such as fruit drinks may require special technology, the combination of probiotic strains with the fruity taste and healthy image of juices has been very successful commercially (Saxelin, 2008). In all cases, precautions have to be taken regarding sensory characteristics and pH of the final product. The latter is important because it has been shown that probiotic viability is reduced during storage in many fermented milks having pH values between 4.0 and 5.0 (Champagne, 2009).

For a microorganism to be designated as a probiotic, the Codex Alimentarius standard for fermented milks requires that the minimum counts of the microorganism, other than starters, at the time of consumption should be 10^6 cfu/g (Codex, 2003). Another requisite of a probiotic is that it needs to reach the target location within the host; that is, the gastrointestinal tract (**GIT**), in sufficiently high numbers to exert a beneficial effect (International Dairy Federation, 1992). Therefore, it is essential that probiotics survive cultivation, concentration, processing, and storage. Moreover, they must survive passage through the GIT. In vivo studies have been performed to assess the survival of yogurt starters during gastrointestinal transit (del Campo et al., 2005; Mater et al., 2005). However, these studies are complex to use for high-throughput screening of bacterial viability. Therefore, we developed an in vitro model to simulate GIT conditions (Fernández de Palencia et al., 2008), which has been applied to analyze β-glucan–producing strains (Fernández de Palencia et al., 2009;

Garai-Ibabe et al., 2010b). Moreover, the study of the tolerance of probiotic bacteria incorporated in different food products to gastrointestinal conditions would be helpful in the selection of a suitable food matrix that contributes to probiotic survival in the GIT (Schillinger et al., 2005).

In this work, we assayed the survival of *P. parvulus* 2.6, *P. parvulus* CUPV22, and *Lb. suebicus* CUPV221 to simulated gastrointestinal conditions. These strains were incorporated into 3 food products (yogurt, orange juice, and juice-milk beverages) with the aim of evaluating the effect of the food matrix on resistance to gastrointestinal stress and their survival during storage conditions. The rheological properties of the new bioproducts were also determined.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Three β-glucan–producing LAB strains isolated from ropy cider were used in this study: *P. parvulus* 2.6, *P. parvulus* CUPV22, and *Lb. suebicus* CUPV221 (culture collection of the Department of Applied Chemistry, University of Basque Country, Spain) and have been previously characterized as potential probiotics (Fernández de Palencia et al., 2009; Garai-Ibabe et al., 2010a). Strains were grown in de Man, Rogosa, and Sharpe (**MRS**, Oxoid, Basingstoke, UK) broth for 48 h at 30°C. Starter cultures *Lb. delbrueckii* and *Strep. thermophilus* were obtained from YoMix (Danisco, Barcelona, Spain). They were grown on yeast glucose lactose peptone (**YGLP**) agar (http://www.cect.org) at 37°C for 48 h for enumeration. For inoculation of food matrices, 1-mL aliquots of an 18-h culture of each LAB strain were centrifuged at $16,000 \times g$ for 3 min, and the pellets were washed with PBS to remove the medium. After a second centrifugation, pellets were resuspended in 1 mL of pasteurized milk or juice and were added to the corresponding food.

Preparation of Yogurt, Orange Juice, and Juice-Milk Beverages

Yogurt Manufacturing. For yogurt manufacturing, semi-skimmed, powdered milk 9% was reconstituted in sterile tap water and the procedure used in Fiszman and Salvador (1999) was followed. Milk was supplemented with 20 g/L of glucose syrup (Cargill, Barcelona, Spain) for *Lb. suebicus* or 20 g/L of glucose and fructose syrup (Cargill) for *P. parvulus*. The mixture was pasteurized at 85°C for 30 min, cooled to room temperature, and inoculated with 2.5 g/100 L (10^6 cfu/mL) of the commercial starter (YoMix, Danisco) and 1

mL of the bacterial culture described previously. One hundred milliliter aliquots of both inoculated milk and control yogurts were placed into a water-bath at 42°C. The pH was measured every 30 min and when it reached a value of 4.7 ± 0.1 (approximately 5 h), fermentation was stopped and the yogurts were stored at 4°C for 28 d. Three aliquots were sampled each week for physicochemical and microbiological analyses. In each experiment, a control batch was manufactured simultaneously containing only the YoMix starter.

Orange Juice and Juice-Milk Beverage Inoculation. Orange juice (**OJ**) was supplied by J. V. Carbonell (IATA, Valencia, Spain). It was prepared immediately, according to Gómez et al. (2009) from fresh Clementine mandarins (*Citrus reticulate* 'Nules'). Two juice-milk beverages were used in this study, a commercially available juice mixture (**CAB**; Pascual Pacífico, Burgos, Spain) and a homemade OJ-milk beverage (**OJMB**). The CAB contained 42.5% of fruit juices (apple, kiwi, orange, and lime juice made from concentrate), 10% skim milk, water, sugar, dextrose, pectin flavor, vitamins C, E, and A, sucralose, chlorophyll, and turmeric extracts with a pH of 3.8. The OJMB was prepared as described previously (Sampedro et al., 2009) and contained the following ingredients: fresh orange juice (500 mL/L), commercial UHT skim milk (200 mL/L), 3 g/L high methoxyl citrus pectin (Unipectine AYD 250, Cargill, Minneapolis, MN), sucrose (75 g/L), and deionized water (300 mL/L) with a final pH of 4.3. For all 3 beverages, 50-mL portions were dispensed into sterile 100-mL glass bottles, and then inoculated with 5 mL of a fresh cell suspension of each β-glucan–producing LAB at a final concentration of 10^8 cfu/mL. A noninoculated control of each beverage was included. Inoculated beverages and negative controls were stored in the dark, under aerobic conditions, at 4°C.

Physical and Microbiological Analyses

Syneresis and Texture. Whey that separated from yogurts was removed using a syringe to calculate the syneresis index (mL per container; Salvador, 1999). Texture tests were performed on yogurts using a TA.XT.plus Texture Analyzer (Stable Micro Systems, Godalming, UK). To determine the gel force, a 20-mm penetration was made with a 1.27-cm diameter probe with a flat base (TA/0.5) at a speed of 1 mm/s. The following parameters were recorded: firmness, as the force at breaking (N), defined as the first significant discontinuity produced in the curve as the plunger penetrated the gel during a total displacement of 20 mm; and the distance (mm) at which breaking took place (Fiszman and Salvador, 1999). Complete profiles of the

curves were also recorded, to compare the slopes and check for typical behavior patterns.

Color. A Konica Minolta CM-3500 spectrocolorimeter (Konica Minolta Sensing Inc., Osaka, Japan) was used to measure the color of the juices. The results were expressed in accordance with the CIELAB system (D65 illuminant and 10° viewing angle). The measurements were made in transmittance mode using 2-mL plastic cuvettes. The parameters measured were L* [L* = 0 (black), L* = 100 (white)], a* (+a* = red), and b* (+b* = yellow). The L* value is an approximate measurement of lightness, which is the property whereby each color can be assigned a value equivalent to a position on the gray scale, between black and white, taking values within the range 0 −100, respectively; a* assigns positive values for reddish colors and negative values for the greenish ones; and b* assigns positive values for yellowish colors and negative values for the bluish ones.

Rheology. The flow properties of the batters were studied using a RheoStress 1 controlled stress rheometer (Haake, Karlsruhe, Germany) equipped with a Phoenix II P1-C25P refrigerated circulation bath (Haake) and plate-plate sensor geometry (60 mm in diameter) with a 0.5-mm gap. A continuous ramp was applied and apparent viscosity was measured as a function of shear rate over the range from 0.5 to 100 s^{-1}, taking 100 points linearly in time at 25°C. Two replicates of each flow curve were run with good reproducibility (the differences between duplicates were <10%).

Sensory Analysis. Twelve panelists evaluated the flavor of orange juices, noting differences between the noninoculated stored control sample and each of the inoculated stored samples following a paired-comparison protocol. In addition, they indicated the preferred OJ of each pair of samples evaluated. Results were analyzed using the corresponding table following a unilateral hypothesis (Roessler et al., 1978).

Viability of LAB in Yogurt, Orange Juice, and Juice-Milk Beverages. Initial microbiota of beverages was determined by plate counts on MRS and tryptone soy agar (Conda, Madrid, Spain). Counts of LAB were performed on 3 inoculated yogurts and 3 control yogurts on the day after fermentation (d 0) and once a week during the shelf-life of the product (28 d). One gram of each yogurt was resuspended in sterile saline solution (0.8% NaCl), serially diluted, and plated onto MRS and YGLP agar. β-Glucan producers were enumerated on MRS agar incubated at 30°C for 48 to 72 h. Starter cultures were enumerated on YGLP agar incubated at 37°C for 48 h. Colony morphology allowed differentiation between *Strep. thermophilus* (white and irregular) and *Lb. delbrueckii* (transparent).

Viable counts and pH measurements were carried out every week for CAB and after inoculation (d 0) and at

the end of the storage (d 28) for OJ and OJMB. Viable cell counts were obtained on MRS agar at 30°C for 48 h. pH measurements were performed using a pH meter (MicropH 2001, Crison, Barcelona, Spain).

Gastric and Gastrointestinal Transit Tolerance Assays

Pure cultures and inoculated foods were subjected to stress conditions simulating the human gut by using an in vitro model, adapted from Fernández de Palencia et al. (2008), which approximates exposure to saliva, the pH gradient of the stomach, and the intestinal stress. The test was done 24 h after inoculation using 20 mL of inoculated food or pure cultures: 2.5 mL was used as a control (sample G1) and the remaining 17.5 mL was challenged with lysozyme 0.01%, pepsin 0.3% and 2.3 mL of electrolytic solution G (6.2 g/L NaCl, 2.2 g/L KCl, 1.2 g/L NaHCO$_3$) and adjusted to pH 5 with 1 M HCl. At this point, 2.5 mL was taken and designated as sample G2. The remaining mixture was distributed into six 2.5-mL aliquots and the natural pH change of the stomach was simulated by lowering the pH by using 1 M HCl from the initial pH 5 (sample G3, 2 aliquots) to pH 4.1 (sample G4), pH 3 (sample G5), pH 2.1 (sample G6), and pH 1.8 (sample G7). All aliquots were incubated at 37°C for 20 min to mimic normal gastric emptying. Gastrointestinal stress (**GI**) was mimicked using one of the G3 samples. To that aim, pH was adjusted to 6.5 with 1 M NaHCO$_3$. Then, the samples were treated with 200 µL of electrolytic solution ID (5 g/L NaCl, 0.6 g/L KCl, and 0.3 g/L CaCl$_2$), 4% bile salts, and 2% pancreatin at 37°C for 120 min to simulate conditions of the duodenum (sample GI3). To estimate cell viability after gastric stress (**G**) and GI, both untreated (G1 control samples) and treated samples from pure cultures, yogurt, and the 3 inoculated drinks were analyzed. Samples were serially diluted in PBS buffer pH 7.5 (10 mM Na$_2$HPO$_4$, 1 mM KH$_2$PO$_4$, 140 mM NaCl, 3 mM KCl) and plated in triplicate onto MRS and YGLP agar.

Statistical Analysis

One-way ANOVA was performed on yogurt and OJ instrumental measurements. Least significant differences were calculated by the Tukey test using the XlStat 2009 software (Addinsoft, Barcelona, Spain).

RESULTS AND DISCUSSION

### Viability of LAB Strains in Yogurt, Orange Juice, and Juice-Milk Beverages

Many LAB strains can grow in milk but usually their growth rates are slower than those of commercial yogurt starters (Champagne, 2009). Therefore, in this work, LAB strains were inoculated at concentrations of 10^7 to 10^8 cfu/mL to obtain populations similar to those of the commercial inocula at the end of fermentation. In yogurts, no differences were found between control and inoculated yogurts in terms of fermentation time within a range of 4 h 45 min to 5 h. However, the pH of the inoculated yogurts decreased slightly faster than that of the control (only commercial starter). Table 1 shows plate counts in the initial cultures used as inoculum, after fermentation (d 0), and after 28 d of storage. None of the added β-glucan–producer strains increased in plate counts after fermentation. Figure 1A shows the evolution of the LAB population of both starter cultures and the added β-glucan producers during the shelf life of the manufactured yogurts. Addition of β-glucan–producing LAB strains had no effect on the growth of the yogurt starters. However at the end of the storage period, LAB populations were 3×10^6, 1.1×10^5, and 5×10^4 cfu/g for *Lb. suebicus* CUPV221, *P. parvulus* 2.6, and CUPV22, indicating a decrease in viability of 1, 2, and 3 log orders, respectively.

As an alternative to dairy products, fruit drinks have been proposed as probiotic carriers (Champagne et al., 2008; Champagne and Gardner, 2008). In this work, a commercially available multijuice-milk beverage, an orange-milk mixture, and an orange juice were evaluated. Juice and juice-milk beverages were inoculated with 10^8 cfu /mL of each LAB strain except *Lb. suebicus*, which was inoculated at 10^5 cfu/mL because of its low growth rate (Table 1). These concentrations correspond to 2.5×10^{10} cfu in a 250-mL portion, which is a commercially representative level of juice supplementation and has been successfully used in previous studies of probiotic-carrier juices (Champagne et al., 2008; Champagne and Gardner, 2008). The pH of the inoculated drinks did not vary during the 28 d of storage and was 3.9 ± 0.1 for OJ, 3.7 ± 0.2 for CAB, and 4.1 ± 0.1 for OJMB. Noninoculated samples showed total counts of 10^2 cfu/mL (contaminant pigmented yeast and bacteria) at the end of the storage period and did not appear on the plates corresponding to inoculated foods. These values represent 0.001% of the total population in inoculated samples, which are too low to interfere with the counts of inoculated strains. Figure 1B shows the plate counts of the inoculated strains during the storage. Little variation was found in the survival of *P. parvulus* strains, which maintained the initial levels (10^7 to 10^8 cfu/ mL) during storage. *Lactobacillus suebicus* CUPV221 showed a 2-log decrease after inoculation, and plate counts decreased 1 log during the storage period in CAB. Plate counts did not vary during storage in OJ and OJMB. Orange juice and OJMB are better food

Table 1. Lactic acid bacteria counts (cfu/g) in the inoculum, after inoculation (d 0), and after storage (d 28)

Species/strain	Inoculum	Yogurt		Commercially available juice-milk beverage		Orange juice		Orange juice-milk beverage	
		d 0	d 28	d 0	d 28	d 0	d 28	d 0	d 28
Pediococcus parvulus 2.6	6.3×10^7	1.2×10^7	1.1×10^5	3.7×10^7	1.9×10^8	8.1×10^7	7.5×10^7	5.7×10^7	5.7×10^7
Pediococcus parvulus CUPV22	1.42×10^7	1.4×10^7	5×10^4	1.5×10^8	9.2×10^7	5.3×10^7	2.2×10^7	5.6×10^7	5.2×10^7
Lactobacillus suebicus CUPV221	1.23×10^7	4.8×10^7	3×10^6	8.4×10^5	5.5×10^4	8.4×10^5	5.9×10^5	2.2×10^5	4.9×10^5
Streptococcus thermophilus	2.7×10^6	1×10^9	8×10^7	—	—	—	—	—	—
Lactobacillus delbrueckii	4.7×10^6	1.2×10^8	9.1×10^6	—	—	—	—	—	—
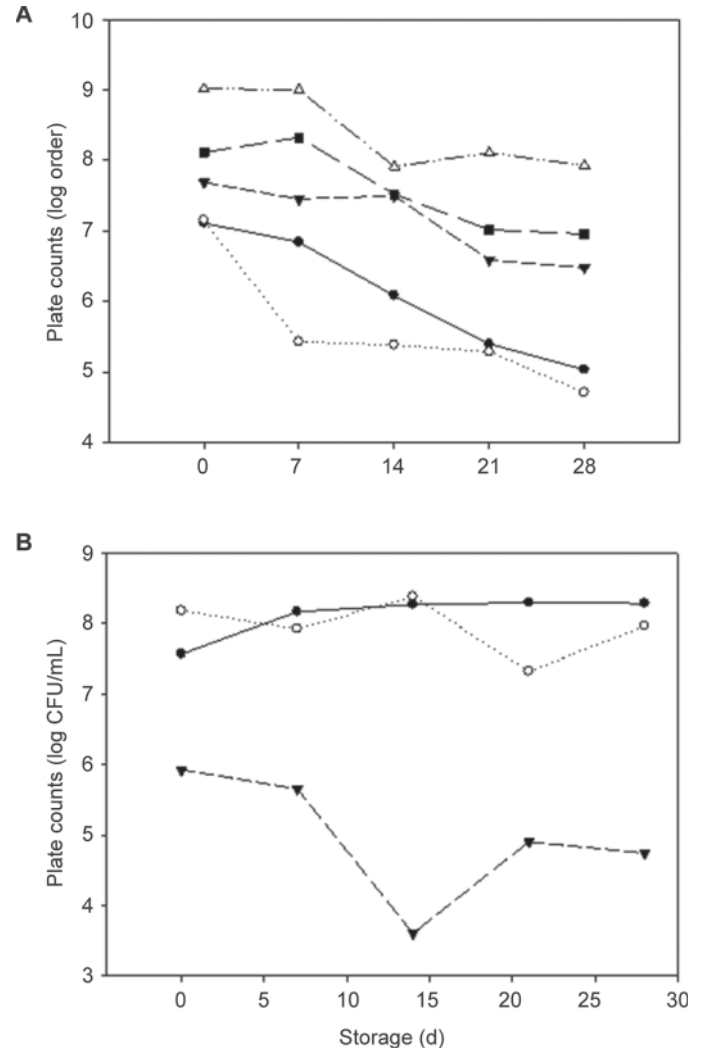
Figure 1. Survival of inoculated strains and yogurt starters in yogurt (A) and commercially available juice-milk beverage (B) during the 28 d of storage: (●) *Pediococcus parvulus* 2.6, (○) *Pediococcus parvulus* CUPV22, (▼) *Lactobacillus suebicus* CUPV221, (Δ) *Streptococcus thermophilus*, and (■) *Lactobacillus delbrueckii*.

carriers for *P. parvulus* strains, whereas yogurt is better for *Lb. suebicus.*

Physical Analysis of Yogurts and Beverages

The results of the physical analysis of the yogurts are shown in Table 2. Texture was evaluated in terms of firmness and breaking distance. Statistical analysis showed that no significant differences in texture were found between the inoculated and noninoculated yogurts, even after 28 d of storage. In terms of syneresis, when comparing each inoculated sample with the control, no significant differences were found. No differences in flow properties were found between inoculated beverages in comparison with the noninoculated bever-

Table 2. Results of texture and syneresis tests performed to inoculated yogurts during the 28-d storage period[1]

Storage time (wk)	Inoculated strain	Firmness (N)	Breaking distance (mm)	Syneresis (mL)
0	Control	7.3 (0.3)[a]	2.2 (0.6)[a]	3.1 (0.6)[a]
	Lactobacillus suebicus CUPV221	10.6 (0.2)[a]	2.0 (0.1)[a]	1.7 (0.6)[a]
	Pediococccus parvulus 2.6	9.0 (1.0)[a]	1.9 (0.2)[a]	6.5 (3.5)[a]
	Pediococccus parvulus CUPV22	7.7 (0.2)[a]	1.6 (0.1)[a]	3.7 (0.1)[a]
1	Control	8.7 (0.3)[a]	2.0 (0.4)[a]	2.5 (1.3)[a]
	Lb. suebicus CUPV221	11.5 (0.6)[a]	1.8 (0.1)[a]	1.6 (0.5)[a]
	P. parvulus 2.6	8.7 (0.7)[a]	1.7 (0.5)[a]	4.5 (1.4)[a]
	P. parvulus CUPV22	9.4 (0.1)[a]	1.9 (0.1)[a]	2.9 (1.6)[a]
2	Control	7.9 (0.4)[a]	2.7 (1.1)[a]	1.9 (0.5)[a]
	Lb. suebicus CUPV221	11.3 (0.9)[a]	2.1 (0.1)[a]	2.2 (0.5)[a]
	P. parvulus 2.6	8.9 (0.1)[a]	2.1 (0.6)[a]	4.1 (0.4)[a]
	P. parvulus CUPV22	9.6 (0.2)[a]	1.9 (0.1)[a]	2.0 (0.4)[a]
3	Control	8.3 (0.1)[a]	2.0 (0.7)[a]	3.9 (1.4)[a]
	Lb. suebicus CUPV221	12.0 (0.6)[a]	1.8 (0.1)[a]	2.1 (0.4)[a]
	P. parvulus 2.6	9.2 (0.1)[a]	1.9 (0.5)[a]	2.0 (0.0)[a]
	P. parvulus CUPV22	9.6 (0.3)[a]	1.8 (0.2)[a]	2.1 (0.3)[a]
4	Control	8.3 (0.4)[a]	1.7 (0.6)[a]	2.2 (0.9)[a]
	Lb. suebicus CUPV221	12.4 (0.7)[a]	1.9 (0.2)[a]	1.6 (0.4)[a]
	P. parvulus 2.6	9.8 (1.5)[a]	2.7 (1.1)[a]	2.5 (2.1)[a]
	P. parvulus CUPV22	10.1 (0.3)[a]	1.9 (0.1)[a]	1.9 (0.1)[a]

[a]Identical superscript letters indicate no significant differences with the noninoculated control.

[1]Numbers in parentheses indicate standard deviations of triplicates.

ages. Table 3 shows results of the physical properties of juices. The evolution of viscosity with shear rate was similar in all the samples studied. Similarly, few differences were found in color determination. Noninoculated samples presented higher L* values and lower b* values than inoculated samples, thus inoculated samples were more yellowish. No significant differences were found in a* values. With respect to sensory analysis, significant differences were found between the noninoculated stored control and each of the inoculated beverages, comparing the results obtained in the paired-comparison discriminatory tests. However, when the results of the paired-comparison preferred test were analyzed, no significant differences in preference were found. Thus, inoculated beverages were preferred equally to control stored ones.

Gastric and Gastrointestinal Transit Tolerance

Besides survival in the carrier food during storage, the probiotic strains also have to survive gastrointesti-

nal transit, and the food matrix may contribute to this (Schillinger et al., 2005). With this aim, the protective effect of the food was evaluated in vitro, simulating the gastrointestinal tract conditions, using both pure cultures and the inoculated food samples. Figure 2 shows cell survival after G and GI stress for the inoculated LAB strains in pure cultures or incorporated into yogurt, OJ, or OJMB. Samples containing lysozyme and pepsin in the electrolytic solution G (G2) did not significantly affect the viability of *P. parvulus* and *Lb. suebicus* strains in any of the food matrices. In pure culture, *P. parvulus* CUPV22 and *Lb. suebicus* CUPV221 survived G down to pH 3, although their viability was markedly reduced from 7.5×10^9 and 9.3×10^9 cfu/mL to 1.5×10^2 and 9.5×10^3 cfu/mL, respectively. They were also recovered after GI at 2.5×10^2 and 60×10^2 cfu/mL, respectively. *Pediococcus parvulus* 2.6 in pure culture was recovered at 4.5×10^2 cfu/mL after G at pH 2.1 but it did not survive to GI. However, its survival increased when it was incorporated in yogurt

Table 3. Results of physical properties of orange juices after 28 d of storage[1]

Sample	Apparent viscosity (Pa·s)	Color parameter[2]		
		L*	a*	b*
Control	0.014[a] (0.001)	100.00[a] (0.78)	0.96[a] (0.07)	2.77[a] (1.31)
Lactobacillus suebicus CUPV221	0.016[a] (0.002)	98.56[b] (0.06)	0.90[a] (0.01)	5.58[b] (0.07)
Pediococcus parvulus 2.6	0.022[a] (0.001)	98.30[b] (0.01)	0.92[a] (0.02)	5.71[b] (0.03)
Pediococcus parvulus CUPV22	0.019[a] (0.001)	98.27[b] (0.03)	0.94[a] (0.01)	5.32[b] (0.03)

[a]Identical superscript letters indicate no significant differences with the noninoculated control.

[1]Numbers in parentheses indicate standard deviations of triplicates.
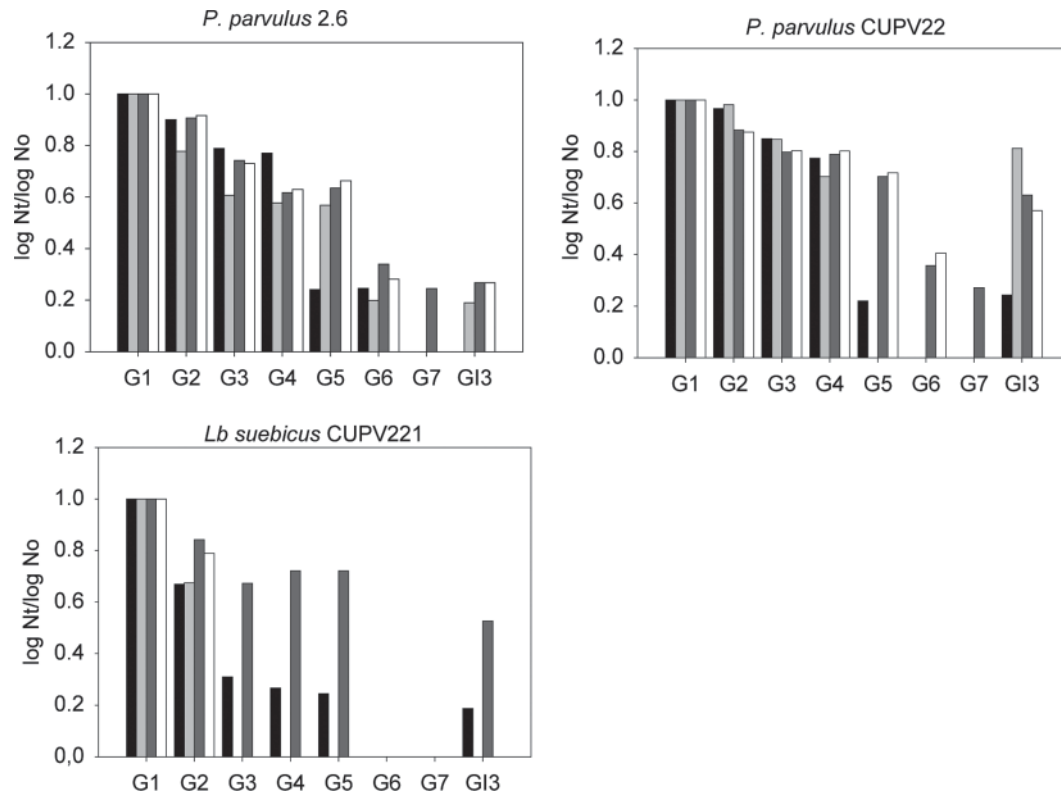
[2]L* = lightness; a* = redness; b* = yellowness.

Figure 2. Analysis of cell survival after gastric stress. The indicated bacterial strains were subjected to various G-stresses (G1, G2, G3, G4, G5, G6, G7) and GI (GI3), as described in Materials and Methods. Plate counts (on de Man, Rogosa, and Sharpe) of the inoculated strains in pure culture (black bars) and inoculated in yogurt (light gray bars), orange juice (OJ; dark gray bars), and orange juice-milk beverage (OJMB; white bars). No = initial bacterial count (cfu/mL); Nt = bacterial count (cfu/mL) after treatment.

and OJMB, where it was recovered after pH 2.1 at 1.25 $\times\ 10^2$ cfu/g and 5.75×10^2 cfu/mL, respectively. It survived to G pH 1.8 in OJ at levels of 2.7×10^2 cfu/mL. *Pediococcus parvulus* CUPV22 was recovered after GI in all food matrices tested and it survived to lower pH in G when inoculated in OJ with plate counts of 5.10×10^2 cfu/mL. *Lactobacillus suebicus* CUPV221 showed a higher G and GI survival rate when inoculated in OJ but was not recovered at the lower pH (2.1 and 1.8); this strain did not survive to G and GI when incorporated into yogurt and OJMB. Previous studies demonstrated that a low pH during bacterial growth could induce a higher acid tolerance (Lorca and Valdez, 2001). Thus, strains inoculated into orange juice, which maintains a pH around 3.8, better resisted the acid challenge of the simulated GI stress. In conclusion, orange juice, besides keeping the strains viable, improves their resistance to gastrointestinal stress; therefore, we propose the use of this highly popular drink as a food carrier for these potentially probiotic strains.

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