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VIABILITY KINETICS OF FREE AND IMMOBILIZED *Bifidobacterium bifidum* IN PRESENCE OF FOOD SAMPLES UNDER GASTROINTESTINAL *in vitro* CONDITIONS

CINÉTICAS DE VIABILIDAD DE *Bifidobacterium bifidum* LIBRE E INMOVILIZADO EN PRESENCIA DE MUESTRAS ALIMENTICIAS BAJO CONDICIONES GASTROINTESTINALES *in vitro*

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Abstract The objective of this work was to study the viability kinetics by a mathematical model of exponential decay of *B. bifidum* un-immobilized (free) and immobilized with sodium alginate under simulated gastrointestinal conditions in presence of food samples. The results demonstrated the protective effect of immobilization support in all experiments, compared to the free bacteria ($p<0.05$). The greater viability was observed in immobilized bacterial cells in the presence of breakfast model (14.7%), on the other hand greater viability loss of *B. bifidum* caused by beer and chili (0% after 30 min simulation) was observed. This study provides useful information on the kinetics of viability loss of immobilized bacterial cells through the upper gastrointestinal tract simulation and the effect of food samples on the viability of probiotic bacteria was demonstrated.

Keywords: *Bifidobacterium bifidum*, gastrointestinal tract simulation, cell immobilization, food samples.

Resumen

El objetivo de la presente investigación fue estudiar las cinéticas de viabilidad por medio de un modelo matemático de decaimiento exponencial donde se evaluó *B. bifidum* libre e inmovilizada con alginato de sodio (mediante la técnica de atrapamiento), tratado bajo condiciones gastrointestinales simuladas *in vitro* en presencia de varias muestras de alimentos. Los resultados evidenciaron el efecto protector del soporte de inmovilización en todos los experimentos, comparado con las bacterias libres ($p<0.05$). La mayor viabilidad se observó en las bacterias inmovilizadas en presencia de desayuno modelo (14.7%), por otro lado se observó mayor pérdida de viabilidad de *B. bifidum* causada por la cerveza y chile (0% después de 30 min de simulación). Este estudio proporciona información útil sobre la cinéticas de pérdida de viabilidad de *B. bifidum* inmovilizada durante su paso por el tracto gastrointestinal superior y el efecto de las muestras de alimentos sobre la viabilidad de las bacterias probióticas fue demostrado.

Palabras clave: *Bifidobacterium bifidum*, simulación del tracto gastrointestinal, inmovilización cellular, muestras de comida.

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1 Introduction

Probiotics are live microorganisms when supplied in adequate amounts confer health benefits to the host and improve the intestinal microflora (Champagne & Fustier, 2007), but to get the benefits probiotics must survive through the gastrointestinal tract (GIT) conditions and implant on the human colon.

Some *Bifidobacterium* and Lactobacillus species are considered probiotic microorganisms, *Bifidobacterium* spp. are lactic acid producers, anaerobic, although some strains can tolerate oxygen; no capsule and nonspore-forming, nonmotile, and nonfilamentous, Gram-positive bacteria that inhabit the intestinal tracts of humans and animals (Meile *et al*., 2008). Bifidobacteria are active in bile acid deconjugation, catabolism of dietary carbohydrates and synthesis of vitamins and are one of the most relevant probiotic microorganisms since they colonize the intestinal tract soon after birth and are present at high population levels in both infants and adults (Rozada-Sánchez *et al*., 2008). Thus, probiotics are known to beneficially modulate several host cell functions, the most prevalent of which are immune responses and intestinal barrier integrity.

Bifidobacterium are mainly administered in combination of food and are widely used as probiotics in functional food products; however, they are sensitive to several factors during processing and its ingestion; additionally probiotics need to adapt to the competitive and changing environment in the human GIT to maintain viability (Reimann *et al*., 2011). The events underlying healthy effects are now beginning to be understood mainly from in vivo and *in vitro* studies of host intestinal epithelial cell or immune cell responses to probiotic strains (Papadimitriou *et al*., 2015).

Among the milk products, yogurt and lacto-fermented beverages are regarded as the quintessential vector for probiotic delivery to the consumer, however to maintain a high number of viable bacteria it is important to select resistant strains to low pH values and effects from bile secretion (Begley *et al*., 2005); the delivery of viable bifidobacteria in the large intestine, where they are able to work (site of action) is limited by the acid conditions of the stomach and by the presence of bile (Aoudia *et al*., 2016). Actually, the benefits of probiotics in humans are well known and it is important to develop systems or models that allow the understanding of the viability bacteria in the GIT. Lactic acid bacteria, such as Bifidobacteria, entrapped or immobilized in a polymeric matrix also could effectively increase its tolerance to gastric juice and bile salts and improve its survival and activity in human intestinal tracts (Ocampo & Carter, 2011). In this regard, cell immobilization technology may provide the necessary safeguard for probiotics (Doherty *et al*., 2010) to impart appropriate entrapment, binding, adsorption, encapsulation and cross-linking to the microorganism and create some kinds of spatial limitation in order to make effective application (Heidebach *et al*., 2010; Ledezma-Delgadillo *et al*., 2016; Pérez-Alonso *et al*., 2015; Salazar-Leyva *et al*., 2014) and also to protect them against the GIT, when they are ingested through fermented milk, ice cream, yogurt (Ainsley Reid *et al*., 2005).

Among the numerous immobilization supports, only a few are considered suitable for food production. For example, inorganic materials are usually excluded because they are characterized as unsuitable for human or animal nutrition. Instead, biopolymers and natural supports of food-grade purity are preferable (Mitropoulou *et al*., 2013) such as alginate, which is generally recognized as safe (FAO, 2001).

The immobilization spheres must be stables and maintain their integrity throughout the GIT until it reaches its site of action (colon), where the immobilization spheres have to release its content. The immobilization support material must retain bacteria and it is also desirable to withstand the adverse conditions presented through the digestive tract.

As aforementioned, the consumption of probiotics brings benefits to the host´s health, however, to get these benefits, probiotics need to be consumed accompanied with functional foods that improve or could be able to maintain the viability throughout the GIT. However, there are some foods that can reduce or inhibit the viability of probiotics such as chili (Careaga *et al*., 2003), sauces and some other foods can maintain the viability (Pacheco *et al*., 2010), for that reason, studies on the survival of probiotic bacteria are important for the development of new probiotic products and also for a better understanding of the possible effects and role of the food involved in the beneficial effects of these microorganisms. Therefore, the aim of this work was to study the viability kinetics of free bacterial cells (un-immobilized) and immobilized *B. bifidum* under GIT *in vitro* conditions with different food samples, in order to evaluate the effect of food samples on the viability of probiotic bacteria.

2 Materials and methods

2.1 Strain and medium

B. bifidum NRRL B-41410 was obtained from the culture collections of the Bioconversion Department (UPIBI-IPN). *B. bifidum* was grown in liquid Man Rogosa Sharpe (MRS) broth supplemented with 0.5 g/L of L-cysteine (Annan *et al*., 2008). The biomass used in the experiments was obtained after inoculating *B. bifidum* from a Petri plate into a flask containing 100 mL of MRS broth supplemented with 0.5 g/L of L-cysteine and after incubating 24h, 37°C and 180 rpm in an orbital shaking incubator (ESEVE, INO-65OV-7, Mexico). *B. bifidum* was preserved in MRS-cys medium and 20 g/L of agar was added to produce solid medium contained in Petri plates (Mainville *et al*., 2005) and was maintained at 4°C and subcultured monthly on Petri plates prepared from MRS-cys medium. All the reagents were obtained from Sigma Aldrich.

2.2 Bacteria immobilization technique

After inoculating *B. bifidum* in 100 mL of MRS broth supplemented with L-cysteine and after 24 h of incubation the produced biomass was harvested by centrifugation at 8000 rpm for 5 min at 4 °C in a centrifuge (Beckman, J2-MC, USA) and after discarding the supernatant, 3 mL of biomass were mixed with sodium alginate (2% w/v) prepared in deionized distilled water and it was stirred to insure complete dissolution. The solution was used to immobilize bacteria by physical entrapment technique and it was placed in a 10 mL syringe and passed through a peristaltic pump with controlled flow (Gilson, Minipulse 3, France) and it was added dropwise into $CaCl_2$ 0.3 M solution, the agitation and temperature (23°C) were controlled (Taqieddin & Amiji, 2004). Spheres size was monitored measuring the diameter with a digital micrometer (Fowler, IP54, China) and the diameter was of 2.0 ± 0.2 mm.

2.3 Food samples preparation

Four food samples were selected and designed according to a questionnaire (data not shown), where frequency of food consumption, daytime and quantity of consumed probiotic and prebiotic food was asked to Mexican college students. The food samples were prepared as described in Table 1, afterwards they were evaluated with *B. bifidum* (free bacterial cells and immobilized bacterial cells) under GIT *in vitro* conditions.

For the control experiments, free (un-immobilized) and immobilized *B. bifidum* exposed to simulate GI *in vitro* conditions (without food sample) was used.

2.4 Design of gastrointestinal in vitro conditions

The GIT simulation was carried out in two jacketed glass bioreactors of 500 mL which had a glass cover to collocate a pH electrode (Eutech, CON 520, Singapore), a thermometer (HB Instruments, 30592T, USA) and entry ports to deliver the solutions that were used during the simulation experiments. In the bioreactor-1, gastric juices from the stomach were simulated, in the bioreactor-2, pancreatic juices from the duodenum (first section of the small intestine) were simulated (Figure 1).

Table 1. Materials and preparation methods of food samples.

	Food model	Breakfast model	Chili	Beer
Materials	8.5% of soluble solid content (corn starch) 100 mL of pasteurized cow milk	100 mL of pasteurized cow milk 60 g of banana (tabasco) 40 g of commercial oatmeal	1.15 g *capsicum annum* type Serrano chili 100 mL distilled water	100 mL of local beer (Indio, cervecería Cuauhtémoc Moctezuma)
Preparation method	Corn starch was added to pasteurized milk, the final consistency was similar to a lacto-fermented beverage	All the materials were liquefied in a disinfected blender during 3 minutes and were put through sterilization (15 min, 121 °C)	Chili was disinfected and added in water, blended and sterilized (15 min, 121 °C)	The beer bottle was disinfected and 100 mL of beer were poured in a previously sterilized flask

All ingredients were obtained from a local market in Mexico city.

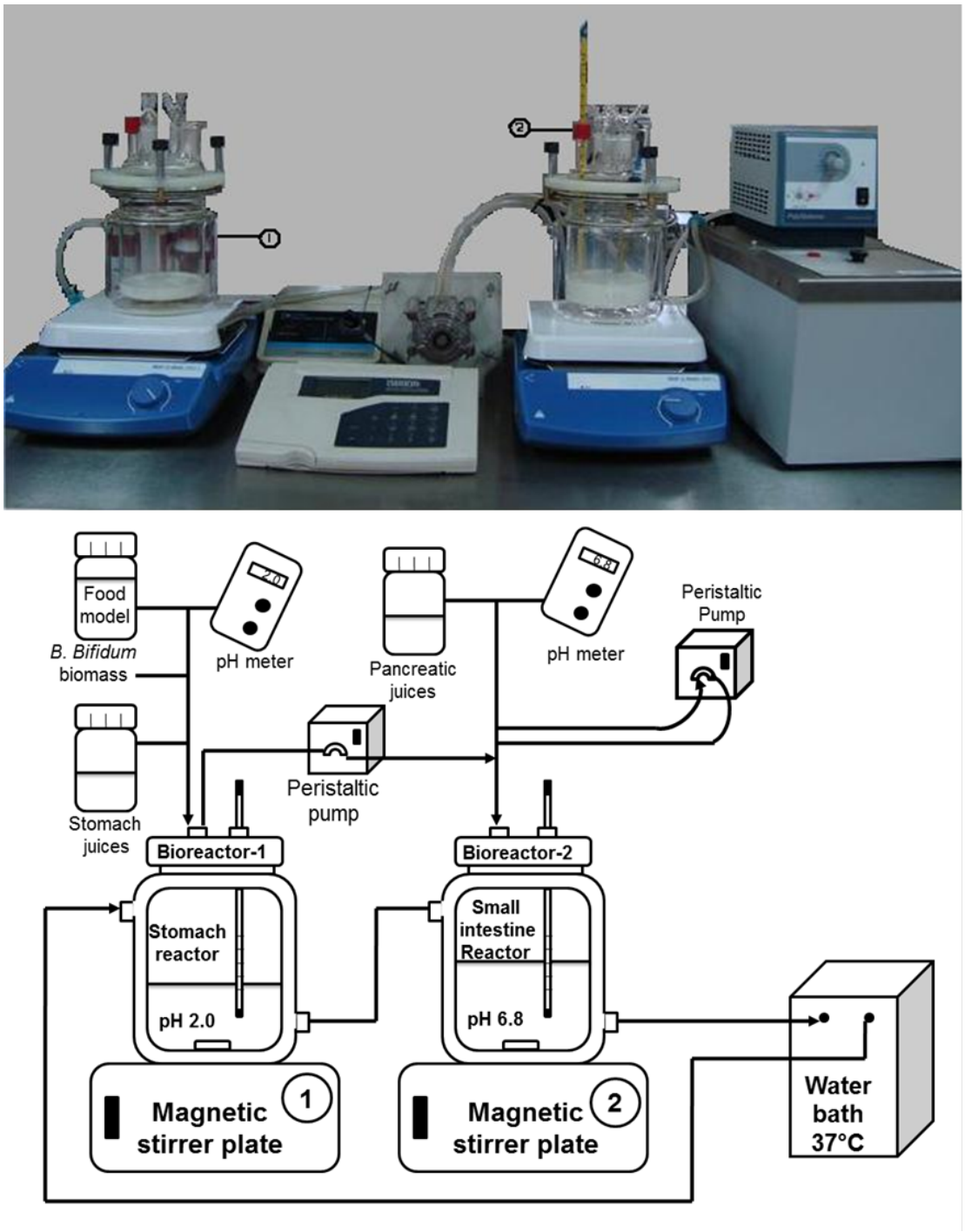

Fig. 1. Scheme of the gastrointestinal tract simulation. Bioreactor-1 simulating gastric conditions (4 g/L of mucin and 3 g/L of pepsin in 50 mL of saline solution, pH 2.0, 100 mL of each food sample, the mixture remained 90 minutes under gastric conditions); Bioreactor-2 simulating intestinal conditions (4 g/L of mucin, 1 g/L of pancreatin and 3 g/L of bile salts in 50 mL of saline solution, pH 6.8, the mixture remained 150 minutes under intestinal conditions).

A magnetic stir bar was located inside each bioreactor and the agitation rate was controlled by a magnetic stirrer plate (IKA, C-MAG MS7, USA) at 50 rpm. The temperature of the process was controlled with a water bath (PolyScience, 812, USA) by circulating water at 37°C through the bioreactors (Mainville *et al*., 2005). The sterility conditions and simulations kinetics were achieved inside a laminar flow bench (Telstar, BV-30/70, Spain). The gastric juices were prepared by dissolving 4 g/L of mucin and 3 g/L of pepsin in 50 mL of saline solution (0.9% w/v), 100 mL of food sample, free (un-immobilized) or immobilized bacterial cells were added to the bioreactor-1, afterwards the pH was adjusted to 2.0 with HCl 5 M. The mixture remained 90 minutes under gastric conditions (Bioreactor-1).The pancreatic juices were prepared by dissolving 4 g/L of mucine, 1 g/L of pancreatin and 3 g/L of bile salts in 50 mL of saline solution (0.9% w/v), the pH was adjusted to 6.8 with NaOH 1.5 M; the mixture remained 150 minutes under intestinal conditions (Bioreactor-2).

2.5 *Viability of free and immobilized bacteria*

The viability of free (un-immobilized) and immobilized *B. bifidum* was determined by taking 1 mL of sample every 30 minutes during 4 hours of simulation (bioreactor-1 and bioreactor-2).

The viability of free bacterial cells was determined from a 1x10-1 dilution (1 mL of sample in 9 mL of sterile saline solution). The viability of immobilized bacteria was measured by dissolving 1 g of immobilized bacterial cells in 9 mL of sodium citrate solution (0.1 M), vortexing (Daigger, Vortex-Genie 2, USA) for 20 s at 37°C (1x10-1 dilution). For free and un-immobilized cells, from the first dilution, 1 mL was taken for the dilution 10^{-2} to 10^{-7} using 0.9% (v/w) sterile saline solution, from each dilution 0.1 mL was inoculated by extension on Petri plates with MRS-cys agar which were incubated during 48 hours and 37°C; the colony forming units per mL (CFU/mL) were obtained by counting on a colony counter (WTW, BZG 30, UK) and the results were reported as viability percentage (% V, see Eq.1).

$$\%V = \frac{CFU_t/mL}{CFU_0/mL} \times 100 \tag{1}$$

Where, CFU_t/mL is the value at time t and CFU_0/mL is the value at initial time. The percentage of viability loss kinetics was calculated every 30 minutes during 240 minutes.

2.6 *Mathematical modeling of viability loss kinetics*

In order to evaluate the viability loss kinetics of *B. bifidum* for each food sample tested as well as the viability of free (un-immobilized) and immobilized bacterial cells, the kinetics data were fitted with a decay exponential model (Eq. 2) and it was selected due to its simplicity and facilitate comparative analysis (Corradini & Peleg, 2004, 2006).

$$V = V_0 + ae^{-kt} \tag{2}$$

Where, k was considered as the rate of viability loss (min^{-1}), viability percentage (V), model constants (V_0 and a), t time (min). Model constants and k coefficients were estimated by using non-linear least squares regression analysis, performed using Marguardt-Levenberg algorithm. The non-linear regression analysis was performed in SigmaPlot software v. 11.0 (SYSTAT Inc. USA). Coefficient of determination (R^2) and the mean relative percent deviation (E_{MD}) (Eq. 3) were used to evaluate the goodness of the fit; R^2 values should be higher (~1.0) and E_{MD} values should be lower (<10.0) (Vega *et al*., 2007).

$$E_{MD} = \frac{100}{N}\sum_{i=1}^{N}\frac{|V_{ex,}) - V_{pre,i}|}{V_{ex,i}} \tag{3}$$

Where, $V_{exp,i}$ is the ith experimental dimensionless viability loss; $V_{pre,i}$ is the ith predicted dimensionless viability loss and N is the number of observations.

2.7 *Statistical analysis*

All experiments were carried out by triplicate and in order to compare different treatments, a two ways ANOVA using Dunett *post hoc* test was conducted using GraphPad Prism 5.0. Unpaired t-test was conducted to compare differences between free and immobilized rate of viability loss.

3 Results and discussion

3.1 *Viability of free (un-immobilized) and immobilized B. bifidum (Control experiments)*

The viability of free and immobilized *B. bifidum* for control experiment presented significant differences ($p<0.05$) between them (Table 2 and Figure 2). The first 90 minutes of kinetics correspond to stomach

conditions while the last 150 min are associated to intestinal conditions. The average of the initial concentration of viable bacteria was 4.73×10^{10} CFU/mL for free and immobilized bacteria that correspond to 100 % of viability. The mucin, pepsin, acid environment (pH 2.0) and the bile salts affected drastically the viability of *B. bifidum*; after 90 min of gastric simulation, the viability percentage decreased 94.2 % (2.74×10^9 CFU/mL) and 83.6 % (7.76x109 CFU/mL) for free and immobilized bacteria, respectively. When bacteria were treated under intestinal conditions (240 min), immobilized bacteria maintained higher viability percentage compared with the free bacteria where the viability percentage for free bacteria was of 1.2 % (5.68×10^8 CFU/ml) and 2.5 % (1.18×10^9 CFU/mL) for immobilized bacteria. According with Mainville *et al.*, (2005), the viability loss of probiotics occurs along the GIT, however the acid environment of the stomach and the bile are factors that mostly affect the viability, bile treatment of bifidobacteria was previously shown to cause a reversible and transient membrane permeabilization which resulted in a loss of viability, as determined by plate counts (Reimann *et al.*, 2011). Additionally, in control experiments for free and immobilized bacteria, k values were of 0.0577 min^{-1} and of 0.0431 min^{-1}, respectively (Table 2), it is was associated with a better protector effect of the immobilization support (Ocampo & Carter, 2011).

3.2 *Viability kinetics of free (un-immobilized) and immobilized B. bifidum under in vitro gastrointestinal conditions*

Figure 2 shows the viability percentage for un-immobilized and immobilized bacterial cells at different times and treatments with food samples. Food model and breakfast model showed significant statistical difference between free and immobilized bacterial cells ($p<0.05$). For immobilized bacterial cells in presence of food model and breakfast model, there was a strong protective effect for the bacterial cells from the damage caused by the external environment which allows the probiotic bacteria to be separated from its environment by a protective coating (Ding & Shah, 2009) and, therefore, this protection maintained the viability of *B. bifidum*. Chili and beer showed an adverse effect in the protection of *B. bifidum*.

Table 2. Decay exponential model parameters of free (un-immobilized) and immobilized *B. Bifidum* (accompanied with food samples) under gastrointestinal conditions.

	Control		Food model		Breakfast model		Chili Beer			
Parameters	Free	Immobilized	Free	Immobilized	Free	Immobilized	Free	Immobilized	Free	Immobilized
k (min^{-1})	0.0577[1]	0.0431[1]	0.0417[2]	0.0300[2]	0.0387[3]	0.0267[3]	0.1018[4]	0.068[4]	0.0561[5]	0.0366[5]
V_0	3.5566	6.1543	1.6176	7.8741	13.8717	16.2328	-0.0308	-0.2281	-0.4657	-0.2281
a	96.3286	92.7130	98.6148	91.3714	85.3785	82.2711	100.0312	100.2528	100.5713	100.2528
R^2	0.9973	0.9832	0.9977	0.9962	0.9849	0.987	0.9999	0.9997	0.9987	0.9997
E_{MD}	9.68	9.91	9.34	4.79	7.33	5.48	0.04	0.32	0.66	2.11

Comparison between free (un-immobilized) and immobilized rate of viability loss (min^{-1}) of meal samples.
p-values [1] 0.008, [1] <0.0001, [3]0.0320, [4] 0.0002, [5] 0.002

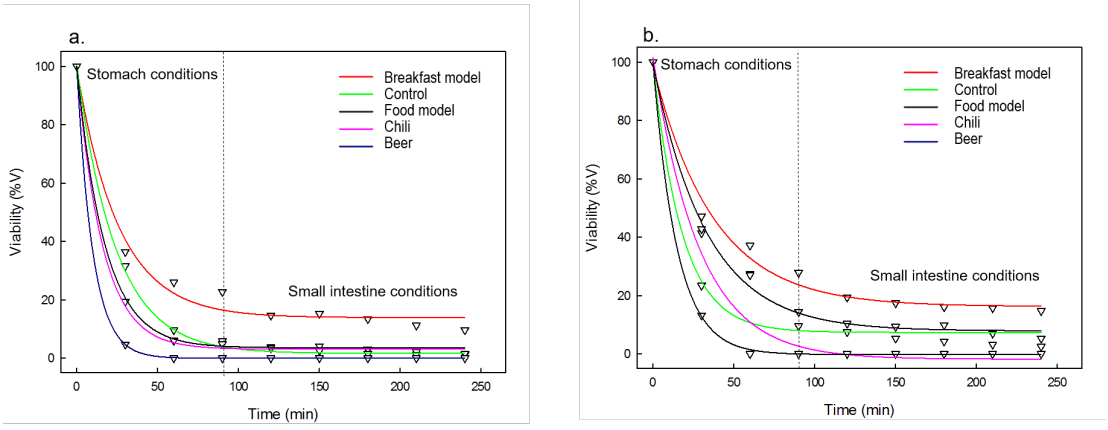

Fig. 2. Modeling of viability loss kinetics (a) free bacterial cells (un-immobilized) and (b) immobilized bacterial cells in presence of food samples.

A typical exponential decay of viability was observed for all kinetics as well as an adequate fitting to decay exponential model (Figure 2 and Table 2). R^2 and E_{MD}, showed a range of values between 0.999-0.982 (R^2) and 9.91-0.04 (E_{MD}). R^2 values greater than 0.98 are accepted as adequate fitting of the experimental data, while EMD values indicates the deviations between the experimental data and predicted line; values lower than 10% are recommended for selection of good fit models (Vega *et al*., 2007).

Additionally, k values were considered as viability loss rate and it was shown that for all cases; k values oscillated between 0.1018 min^{-1} and 0.0267 min^{-1} which were greater in free than immobilized bacteria for control and food samples (Table 2); this fact indicates that the immobilization support provide bacteria protection to simulation conditions. Several authors (Ocampo & Carter, 2011; Pacheco *et al*., 2010; Reimann *et al*., 2011) had demonstrated that this technique provides protection to the bacteria when submitted to similar conditions; also the protective effect can be associated to the immobilization support that provides a barrier to diffusion of gastrointestinal components.

3.3 Viability kinetics of free and immobilized B. bifidum (food samples experiments)

The viability kinetics of free and immobilized *B. bifidum* for food model, breakfast, chili and beer experiments presented significant differences ($p<0.05$) between treatments (Figure 2). The average of the initial concentration of viable bacteria for all experiments was of 4.73×10^{10} CFU/mL (100% of viability). The viability percentage of free *B. bifidum* at the end of the kinetic was of 1.6% (4.57x108 CFU/mL) for food model, 9.6% (4.51x109 CFU/mL) for breakfast, and 0% for chili and beer. The results of viability for immobilized *B. bifidum* after 240 min of kinetic were of 5.3% (2.49×10^9 CFU/mL) for food model, 14.7% (6.91×10^9 CFU/mL) for breakfast, and 0% for chili and beer. In all the experiments, k values were higher in free than immobilized bacteria due to the protective effect of the immobilization support (Table 2).

In food model experiments k value for free bacteria was of 0.0417 min^{-1} and of 0.0300 min^{-1} for immobilized bacteria (Table 2). The immobilization support provided protection for *B. bifidum* from the adverse gastric and intestinal conditions; as a result, higher viability of immobilized bacteria (5.3%) survived after 4 hours of simulation, compared with free bacteria (1.6%), which indicates that the immobilization avoided bacteria damage by their protective effect to *B. bifidum* (Figure 2). Food model promote a higher viability due to k values for food model experiments were lower in comparison with control assays (Table 2), this indicate that the viability loss rate decreased under food model conditions and may be associated with a major viscosity in the medium that could alter the reaction medium and limit the diffusion of the gastrointestinal secretions especially into the immobilization spheres (Ainsley *et al*., 2005).

A better protective effect was found with breakfast model experiments, showing that the immobilization technique together with some food model provided protection for *B. bifidum* against the adverse conditions of the simulation. As a result the highest viability of immobilized bacteria (14.7 %) in comparison with free bacteria (9.6 %) was found (Figure 2). In breakfast model experiments, k values for free bacteria were of 0.0387 min^{-1} and 0.0267 min^{-1} for immobilized bacteria; the k values for breakfast were lower than those obtained in control experiments (free 0.0577 min^{-1} and immobilized 0.0431 min^{-1}, Table 2), this behavior could be associated with a "buffer effect" (Ruas-Madiedo *et al*., 2002). The addition of a food model such as the breakfast sample can have a positive impact on the viability due to dietary factors have been shown to affect bioactive compounds (Reyes-Méndez *et al*., 2015) such as banana (from breakfast model) that contains fructo-oligosaccharides (FOS), a prebiotic that can modify the gut microbiota and their metabolic activities in a beneficial way (Romero-López *et al*., 2015) by stimulating the growth of LAB in the human colon (Ouwehand *et al*., 2005). When combining LAB such as *B. bifidum* with fruits like banana, a synergetic effect can be expected. The addition of breakfast model in the GIT simulation minimizes the loss of bacterial viability because of the fiber content on the oatmeal and FOS of the banana may act as prebiotics, furthermore the immobilization support provides extra protection to *B. bifidum*. Ruas-Mediedo *et al*., (2002) proposed that a food model may have a favorable impact on the viability of LAB by a "buffer effect" and possibly because it provides a protective barrier to adverse conditions.

Chilli and beer model presented negative effect on the viability; the kinetics showed that free bacteria had higher viability loss in comparison with immobilized

bacteria (Figure 2). The rate of viability loss of *B. bifidum* free and immobilized for chili experiments were of 0.1018 min^{-1} and 0.068 min^{-1}, respectively; and the k values for beer were of 0.0561 min^{-1} and 0.0366 min^{-1} for free and immobilized bacteria, respectively. These values were higher than control experiment for free and immobilized bacteria (Table 2).

In chili experiments, *B. bifidum* remain viable only for the first 30 minutes of the kinetic, which was 4.7% (2.22×10^9 CFU/mL) for free bacteria and 13.2% (6.24×10^9 CFU/mL) for immobilized bacteria. After 30 min no microbial count was obtained, it is evident the negative effect of chili on the viability in comparison with control experiment (Table 2). Careaga *et al*. (2003) evaluated the inhibitory effect of the extract of *Capsicum annuum* bell pepper type against bacteria and it was shown that a bactericide or bactericidal effect was produced due to the extract of *C. annum*; capsaicin, caffeic and cinnamic acid are compounds in chili that affect the viability of the bacteria (Acero-Ortega *et al*., 2005), for this reason it is possible that the capsaicin and cafeic acid of the Serrano chilli and the high sensibility of *B. Bifidum* in GIT conditions could be the main factors affecting the viability (Figure 2).

For beer experiments the viability was monitored the first 30 minutes of the kinetic, free bacteria showed 19.4% (9.18×10^9 CFU/mL) of viability while the immobilized bacteria maintained their viability in a 42.8% (2.02×10^{10} CFU/mL), afterwards the viability of *B. bifidum* was of 0%. As seen in Figure 2, the viability is lost completely after 30 minutes under simulated gastric conditions. The bacteriostatic effect of ethanol is well known, this fact implies that when probiotics are consumed with beer or other beverages with a similar content of alcohol (6°G.L) probably there will not be any benefic effect provided by the probiotics because they will not be able to reach their site of action, whether immobilized with a protective polymer or not.

Conclusions

Acid pH in the stomach, bile salts in the small intestine and the presence of some digestive enzymes such as pepsin and pacreatin, contribute to the viability lost. Decay exponential model was successful to evaluate and compare the viability loss rate between free and immobilized bacteria as well as the effect of the food samples. In all experiments the immobilization technique was helpful to maintain the viability; consequently the k values were lower for immobilized than free bacteria. Food model and breakfast model had higher protective effect on the viability than beer and chili samples. Thus, the present study provides useful information about the viability loss kinetics of immobilized *B. bifidum* during upper gastrointestinal tract transit and the effect of food model on the viability of probiotic bacteria was demonstrated.

This research illustrates the importance of incorporating food while probiotic bacteria are consumed, so it is more likely that the bacteria can reach its site of action in sufficient quantities to produce its physiological effect on the host and get the real benefits of probiotics.

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Nomenclature

a	model constant
CFU	Colony Forming Units
CFU_t	CFU at time t
CFU_0	CFU at initial time
E_{MD}	mean relative percent deviation
k	rate of viability loss (min^{-1})
N	number of observations
R^2	coefficient of determination
t	time (min)
V	viability percentage
V_o	model constant
V_{expi}	ith experimental dimensionless viability loss
V_{prei}	ith predicted dimensionless viability loss

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