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Survival of a mixed culture of microencapsulated probiotic strains against the gastrointestinal barrier *in vitro*

Supervivencia de un cultivo mixto de cepas probióticas microencapsuladas frente a la barrera gastrointestinal *in vitro*

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ABSTRACT

Encapsulating materials preserve the viability of probiotics under gastrointestinal conditions. The aim of the research was to evaluate the protective effect of an encapsulating matrix, composed for the first time with three prebiotic materials to maintain the viability of a mixed culture of spray–dried microencapsulated probiotics under simulated gastrointestinal and prebiotic conditions. Microcapsules of four formulations with better viability were then evaluated by inoculating microencapsulated and free strains in MRS broth, adjusting three pH values, bile salts, broth with and without carbohydrate (prebiotic test), incubated at 36 ± 1°C / 24 h; then the percentage of post–treatment cell survival was calculated. Showing that, formulation 1 presented higher barrier protection with average counts: 7.31 log CFU·g^{-1} lactobacilli and 7.75 log CFU·g^{-1} (*Saccharomyces boulardii)* / 4 h (SGF), reaching 6.78 log CFU·g^{-1} in the four formulations (SIF) with a higher average survival rate 79.79% and 85.06% SGF and SIF, *in vitro*. On the other hand, the prebiotic test maintained average counts of 9.40 log CFU·g^{-1} (*Lactobacillus* spp.) and 6.99 log CFU·g^{-1} (*S. boulardii)* / 24 h. The protection exerted by the microspheres under simulated gastrointestinal and prebiotic conditions at therapeutic levels (≥ 10^6 CFU·mL^{-1}) was demonstrated.

Key words: Gastrointestinal barrier protection; probiotic strains; prebiotics; survival; microencapsulated strains

RESUMEN

Los materiales encapsulantes conservan la viabilidad de los probióticos en condiciones gastrointestinales. El objetivo de la investigación fue evaluar el efecto protector de una matriz encapsulante, compuesta por primera vez con tres materiales prebióticos para mantener la viabilidad de un cultivo mixto de probióticos microencapsulados por secado por aspersión, bajo condiciones gastrointestinales y prebióticas simuladas. Seguidamente, se evaluaron las microcápsulas de cuatro formulaciones con mejor viabilidad, inoculando cepas microencapsuladas y libres en caldo MRS, ajustando tres valores de pH, sales biliares, caldo con y sin carbohidrato (prueba prebiótica), incubados a 36 ± 1°C / 24 h; luego se calculó el porcentaje de supervivencia celular postratamiento. Demostrando que, la formulación 1 presentó mayor barrera de protección con recuentos promedio: 7,31 log ufc·g^{-1} lactobacilos y 7,75 log ufc·g^{-1} (*Saccharomyces boulardii)* / 4 h (SGF), alcanzando 6,78 log ufc·g^{-1} en las cuatro formulaciones (SIF) con una mayor tasa de supervivencia promedio 79,79% y 85,06% SGF y SIF, *in vitro*. Por otra parte, la prueba prebiótica mantuvo recuentos promedio de 9,40 log ufc·g^{-1} (*Lactobacillus* spp.) y 6,99 log ufc·g^{-1} (*S. boulardii)* / 24 h. Se demostró la protección ejercida por las microesferas en condiciones gastrointestinales y prebióticas simuladas a niveles terapéuticos (≥ 10^6 ufc·mL^{-1}).

Palabras clave: Barrera gastrointestinal; protección; cepas probióticas; prebióticos; supervivencia; cepas microencapsuladas

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INTRODUCTION

Probiotics are live microorganisms that when consumed in adequate amounts colonize in the digestive tract, conferring a health benefit to the host, which is why today the food industry shows a growing interest in the incorporation of probiotic microorganisms for the elaboration of Functional Foods (FA), particularly in fermented dairy products (yogurt, fermented milk and cheese) and non–fermented products such as ice cream [1]. The medicinal or therapeutic efficacy of these foods depends on the number of colony forming units per mL or gram ($CFU \cdot mL^{-1}$ or g) of viable probiotic microorganisms in the product at the time of consumption. The minimum amount recommended by the US FDA (Food and Drug Administration) and the food industry in general was 10^6 $CFU \cdot mL^{-1}$ [2].

According to Avila–Reyes *et al.* [3], the ability of probiotic microorganisms to survive and develop in the host will directly influence their probiotic effects. Probiotic bacteria must be protected from the adverse environment represented by the food matrix and the gastrointestinal tract, in order to avoid a negative sensory impact when they are incorporated into foods, as supplemented, exposed to food conditions that could be adverse or favorable: pH, humidity, temperature, oxygen concentration, water activity, nutrient availability, presence of inhibitors, among others, which can affect their viability and stability during storage and commercialization as functional foods. Therefore, it is necessary to improve preservation and protection conditions that guarantee the integrity of probiotics when they are incorporated and processed in food matrices, as well as to ensure their viability and activity when they are released in the intestine where their action is required [3, 4].

The acid–tolerant capacity of bacteria is one of the common characteristics among microorganisms of the *Lactobacillus* genus [5, 6]. *Lactobacillus plantarum* strains are considered a unique probiotic because of their ability to resist acidic conditions by possessing cellular mechanisms to maintain intracellular pH close to neutrality, withstanding lower pH values than most other microorganisms [5]. On the other hand, it was evaluated the viability of *L. rhamnosus* in relation to the conditions of an acid medium, caused by fermentation, with the resistance of the microorganism to pH close to 4.0, demonstrated that this species produces lactic acid efficiently through carbohydrate intake, preferably using hexoses [7]. The yeast *Saccharomyces cerevisiae* var. Boulardii, has been widely used as a preventive and therapeutic agent in the treatment of diarrhea under the presentation of lyophilized or heat–dried, the Word Gastrointestinal Organization (WGO) recommends consuming 5×10^9 CFU of *S. boulardii* for certain gastrointestinal disorders [8, 9, 10]. The ability of *S. boulardii* yeast to grow over a wide pH range is known [8, 11].

One of the methods to prevent the decrease of cell load and/or damage of probiotic bacteria is encapsulation. Microencapsulation is a process by which bioactive materials are coated with other protective materials or their mixtures as an alternative to maintain high viability of microorganisms, protecting the core material from environmental stress, such as oxygen, high acidity and gastric conditions, and can be used to cross the gastric barrier with little harmful effects. Among different microencapsulation techniques, spray drying is commonly used because of advantages such as low operating costs, high production rates, low moisture content in the final product, and possibility of industrial–scale application [12], involves atomization of the feed solution in the hot air–drying chamber, where water is evaporated from the atomized droplets to form a dry powder [13]. Prebiotics defined as the food of probiotics and generally represented by oligosaccharides and fibers that are not digestible by humans, are resistant to gastric acidity and digestion by small intestine enzymes and provide a fermentable carbohydrate for probiotic bacteria in the colon. High molecular weight β–glycan glucose polymers (polysaccharides) are found naturally in the cell wall of various living organisms such as bacteria, yeasts, fungi and plants (cereals such as oats (*Avena sativa*) and barley (*Hordeum vulgare*)) [14].

Their combination can reinforce each other's effect, hence, the synergistic combination of probiotics and prebiotics, termed "symbiotics", which improves viable counts of lactobacilli and *bifidobacteria* compared to probiotic or prebiotic alone [9, 15]. The addition of prebiotics in the encapsulation of probiotic microorganisms, in what could be defined as a "co–encapsulation", can favor the viability and efficacy of these beneficial microorganisms in the gastrointestinal tract. Some authors have reported that this co–encapsulation can improve the functionality of the immobilized microorganism, generating higher counts compared to encapsulation without prebiotic [16].

Several studies have been carried out on microencapsulation using encapsulating materials such as: sodium alginate, calcium alginate, chitosan, modified starch, maltodextrin, xantha gum, among others [17, 18], achieving the preservation of probiotic microorganisms during food storage and processing; However, they have the disadvantage that all materials are porous to a greater or lesser degree, which allows ion exchange directly affecting the pH inside the capsule and consequently decreases the bioactivity of probiotics, reason why the trend today is to use mixtures of encapsulating materials to enhance and increase the viability of microorganisms improving their protective properties against gastric and intestinal barrier conditions [13, 19].

However, the survival of a mixture of three probiotic microorganisms (two *lactobacilli* and one yeast) microencapsulated by spray drying in a system composed of three prebiotic polymers as encapsulating material subjected to *in vitro* gastric juice conditions has not been evaluated. Therefore, the present research proposed to evaluate the protective effect of a prebiotic encapsulating matrix on the survival of the encapsulated microorganisms, using for the first time a mixture of sodium alginate, native cassava (*Manihot esculenta*) starch and oat flour (β–glycan) against gastrointestinal and prebiotic barriers *in vitro*, as an innovative alternative.

MATERIALS AND METHODS

The work was carried out at: Nanotechnology Laboratory, University of Pamplona, Laboratory of Applied Engineering and Bioprocesses and Fermentations Berastegui headquarters, University of Cordoba, Colombia. A pilot spray dryer, Vibrasec S.A©, model PSALAB 1.5, stainless steel co–current flow, Medellín, Colombia, was used.

Encapsulating agents

The following were used: Native cassava starch. Starch Factory of Sucre SAS, Innovayuca©; Sodium Alginate SQ 942. Trademark Cape Crystal Brand©; Oatmeal. Original Ground Oats, Trademark Quakert ©.

Methods

The population was comprised of 28 mixtures of encapsulating material obtained through the Design expert version 10 program and supplemented with an approximate probiotic microorganism count

of 1 × 10^{12} CFU·mL^{-1}. The sample was made up of four formulations composed of sodium alginate, oat flour (β–glycan) and native cassava starch. In order to define the best formulation to be used in microencapsulation, restrictions were defined: moisture content (3 to 4%), viability (> 8 CFU·mL^{-1}) and viscosity (100 to 250 mPa.s), [3, 4, 20, 21]

The sample consisted of four formulations composed of sodium alginate, native cassava starch and oat flour (β–glycan) in different percentages (%) respectively: formulation 1 (0.49 – 2.13 – 9.38); formulation 2 (0.49 – 9.38 – 2.13); formulation 3 (0.62 –7.90 – 3.48); formulation 4 (0.92 – 10 – 1.08).

The probiotic microorganisms evaluated were: Freeze–dried strains of *Saccharomyces boulardii*, CNCMI – 745, Biocodex SAS©, using YGC broth, Merck©, Sabouraud Dextrose Agar, Acumedia© brand for their counts; Suspension strains of *L. plantarum* JCM1149, isolated strain in the Biotechnology Laboratory; Freeze–dried strains of *L. rhamnosus* GG, Merck©, there were used for counting broth and agar MRS, Scharlau© brand.

The cell viability of free and microencapsulated probiotic microorganisms (four selected treatments) subjected to Simulated Gastric Fluid (SGF), Simulated Intestinal Fluid (SIF), and prebiotics, was evaluated, selecting the treatment with the best resistance to the three *in vitro* conditions.

Evaluation of the *in vitro* viability of microencapsulated probiotic microorganisms to gastric and intestinal fluid barrier conditions

The resistance of free and microencapsulated microorganisms under the condition of SGF and SIF, [22, 23], with modifications. To represent SGF conditions, MRS broth was prepared, supplemented with 1% pepsin by adjusting the pH to 1.0, 2.0 and 3.0 using concentrated HCL [22], To prepare SIF, the amount of bile salts necessary to reach a concentration of 0.1, 0.2, 0.3% (v/v) was dissolved in MRS broth, adjusting the pH to 7.5.

The broths obtained were sterilized according to the manufacturer's instructions. A 1:10 dilution was made from 1 g of the mixture of microencapsulated probiotic strains and 1 mL of a mixture of strains without microencapsulation (control) in 9 mL of peptonized water homogenizing with constant agitation for 30 min. 100 µL of each mixture was inoculated into 10 mL of SGF and SIF, respectively, incubated at 36 ± 1 °C. Counts were performed by serial dilutions at times 0, 1, 2, 2, 3, 4 h to assess cell viability [22]. Percent survival was determined according to the following equation [3]:

$$Percentage\ of\ survival\ (\%) = \frac{\log CFU\ N_1}{\log CFU\ N_0} \times 100 \qquad \text{EC. (1)}$$

Where: N_1 represents the total number of viable cells after the treatments (SGF–SIF) and N_0 represents the initial number of viable cells inoculated before the treatments (SGF–SIF).

Evaluation of the viability of microencapsulated microorganisms submitted under *in vitro* prebiotic conditions

Based on the formulation of the MRS broth, model broths with and without the main carbon source (sucrose) were prepared and 10 mL tubes were prepared, which were inoculated with 100 µL of the 1:10 mixture obtained in the previous methodology and incubated at 36 ± 1°C. Counts of the SGF and SIF cultures were performed by means of serial dilutions at times 0, 1, 2, 3, 4, and 24 h to assess the capacity for cell multiplication. Additionally, the pH and the titratable acidity were determined [3].

All the data were statistically analyzed using a factorial ANOVA, with the viability of the probiotic strains as an independent variable with a confidence level of 95%. It was performing multiple comparisons by Tukey test to establish where there were significant statistical differences between the four formulations and the viability of the mixture of microencapsulated microorganisms.

RESULTS AND DISCUSSION

Evaluation of the *in vitro* viability of microencapsulated microorganisms under gastric, intestinal and prebiotic barrier conditions

The results obtained in the different viability tests under gastric, intestinal and *in vitro* prebiotic barrier conditions are presented below.

Viability of a mixed culture of microencapsulated probiotic strains in a prebiotic matrix under Simulated Gastric Fluid (SGF) barrier conditions *in vitro*

FIG. 1, shows how formulation 1 presents a higher barrier of protection in the three microencapsulated strains subjected to pH (1.0, 2.0 and 3.0), maintaining average counts of 7.31 log CFU·g^{-1} for lactobacilli and 7.75 log CFU·g^{-1} for *S. boulardii* during 4 hours of exposure. While microcapsules of formulations 2, 3 and 4 showed lower barrier protection; however, they maintained counts above the recommended therapeutic level (> 6 log CFU·g^{-1}). At the same time, greater protection was observed in the encapsulated lactobacilli with an average reduction of 1.82 log CFU·g^{-1}, and in the yeast a reduction of 2.05 log CFU·g^{-1}; while the free microorganisms showed an average reduction of 3.52 CFU·mL^{-1} log, demonstrating that the four formulations used with different mixtures as wall material when forming the microcapsules, provide different degrees of protection to the encapsulated strains of *L. plantarum*, *L. rhamnosus* and *S. boulardii* strains.

Several researchers indicate that the production of symbiotic microcapsules with the combination of wall matrix employing prebiotic material (fruit-oligosaccharides (FOS), denatured whey protein isolate DWPI) by spray drying method has potential applications in functional food industry with good results [16, 24, 25].

As shown in TABLE I, formulation 1 composed of sodium alginate (0.49%), native cassava starch (9.38%), oat flour (β–glycan) (2.13%), shows the highest *in vitro* survival rate among the four formulations, maintaining the viability of the mixed culture of three microencapsulated probiotic strains with an average of 79.79% during 4 h of exposure at pH values 1.0, 2.0 and 3.0 in SGF, *in vitro*. On the other hand, formulations 2, 3 and 4 show an average survival rate of 47.030 and 49.10%, being statistically different with respect to formulation 1 and the free microorganism strains ($P<0.05$). On the other hand, formulations 2, 3 and 4 show an average survival rate of 47.030 and 49.10%, being statistically different with respect to formulation 1 and the free microorganism strains ($P<0.05$). It is observed that there are significant differences ($P<0.05$) in the average survival rates at the three pH values evaluated (1.0, 2.0 and 3.0) being higher the average survival rate at pH 3.0 with 63.726, while the type of probiotic strain does not present significant differences in the average survival rate ($P<0.05$) indicating that the strains used have similar behavior against the simulated gastric barrier.

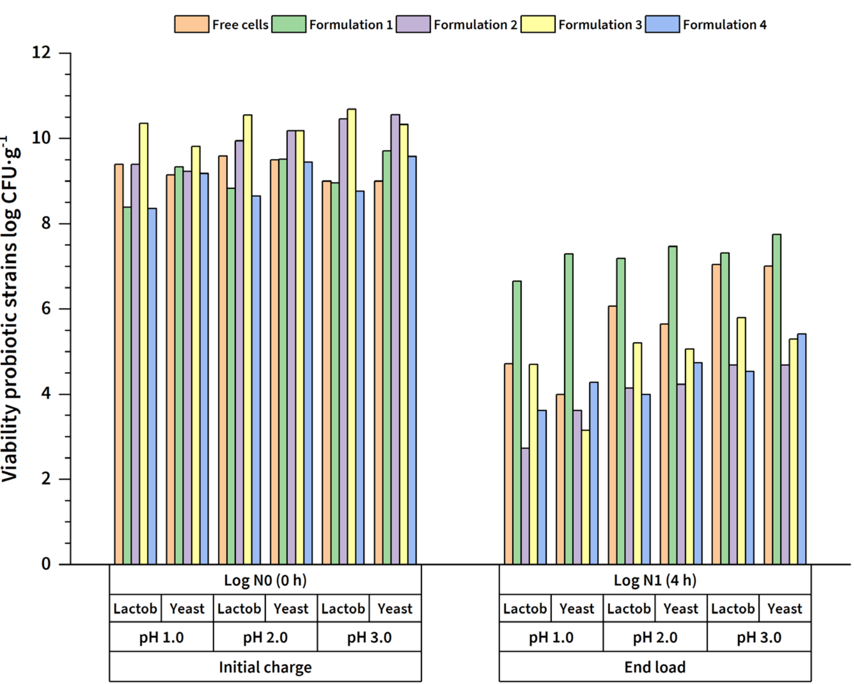

FIGURE 1. Viability of mixed culture of three microencapsulated strains against the Simulated Gastric Barrier (SG) at an exposure time of 4 h. Lactob: *Lactobacillus*

TABLE I
Survival rate of three microencapsulated probiotic strains in a prebiotic matrix versus SGF, *in vitro*

Dependent variable: Microorganism growth (CFU·mL^{-1})

Independent variables	Categories	Mean	*P* value
pH	3	63.726[a]	0.000
	2	58.131[b]	
	1	51.229[c]	
Formulation	Free of microorganisms	65.526[b]	0.000
	Formulation 1	79.790[a]	
	Formulation 2	47.030[c]	
	Formulation 3	47.030[c]	
	Formulation 4	49.100[c]	
Type of strain	*Lactobacillus*	58.241[a]	0.552
	S. boulardii	57.149[a]	

Averages that do not share a letter are significantly different

The above demonstrates that the mixture of polymeric materials used in the four formulations evaluated, offers a protective barrier to gastric simulation conditions *in vitro* because the prebiotic and encapsulating properties are enhanced by combining sodium alginate with oat flour (β–glycan) and native cassava starch, which provide fibre and polysaccharides of high resistance to the gastric barrier, exerting greater protection on the mixed culture of encapsulated probiotic strains [11, 12, 25, 26, 27, 28, 29, 30]. Results that coincide with authors who determined a strong reduction of viability in free cells at pH 2.0 with respect to those microencapsulated by spray drying using various materials (whey protein, milk powder, alginate, chitosan, inulin, among others) conferring greater protection against SGF, *in vitro* [10, 13, 18, 25, 26].

Viability of three strains of microencapsulated probiotic microorganisms in a prebiotic matrix under intestinal barrier conditions SIF *in vitro*

The tolerance of prebiotic microcapsules to the bile salt environment is an important property [31], The viability results of the three microencapsulated strains against the SIF intestinal barrier *in vitro* at an exposure time of 4 h are presented below (FIG. 2).

The results in FIG. 2 show that the three encapsulating materials used in the four formulations tolerate SIF barrier conditions, *in vitro*, at bile concentrations (0.1, 0.2 and 0.3%). The free strains showed a reduction of 1 log CFU·g^{-1}, with lower microbial growth at the bile concentration of 0.3% (7.09 log CFU·g^{-1}); however, the average counts were higher than the required therapeutic values. A decrease of 3.05 log CFU·g^{-1} was observed after 4 h of exposure to barrier conditions (SIF, *in vitro*), reaching a mean value of 6.78 log CFU·g^{-1} in the four formulations, probably due to the protective effect exerted by the wall of the prebiotic microcapsules, results similar to those reported by other authors who have demonstrated *in vitro* the protective capacity of different materials against the SIF barrier [11, 13, 18, 25, 26].

It can be observed in TABLE II that the prebiotic microcapsules of formulation 1 presented statistically significant differences with $P<0.005$, with the highest average survival rate (85.060%) with respect to formulations 2, 3, 4 and the free probiotic microorganism strains, showing significant differences with $P<0.005$ in the three values of the average counts of microorganisms against the percentages

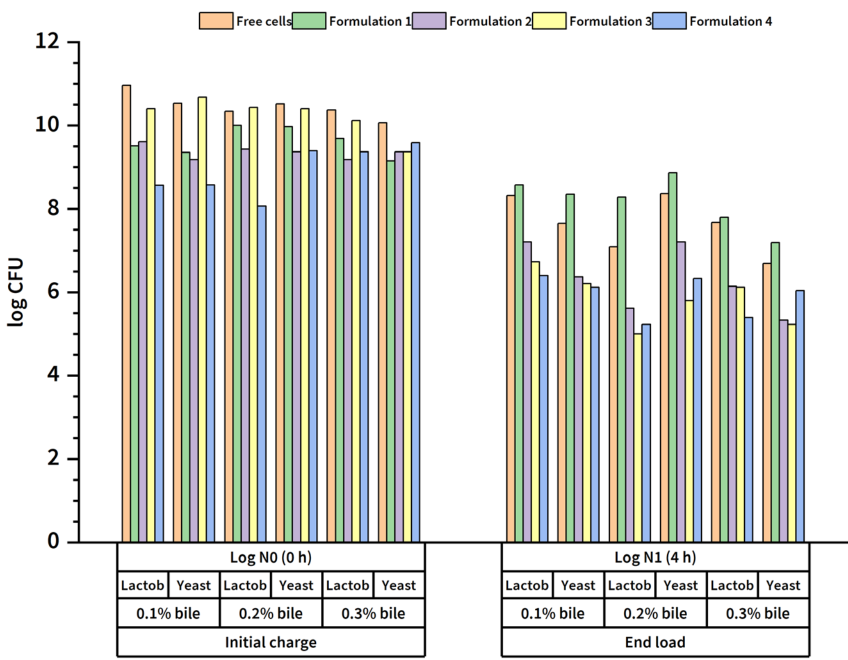

FIGURE 2. Viability of mixed culture of three microencapsulated strains against the intestinal barrier (SIF) at an exposure time of 4 h. Lactob: *Lactobacillus*

of bile evaluated (0, 1, 0.2, and 0.3%) showing that the mixture of encapsulating materials used in formulation 1 offered a better protective barrier against SIF conditions, *in vitro* maintaining the viability of the mixed culture of probiotic strains in the three concentrations of bile after 4 hours of exposure for both lactobacilli and yeast *S. boulardii*.

Likewise, the survival rate according to the type of probiotic strain evaluated showed no significant statistical differences with a $P>0.05$,

TABLE II
Survival rate of three probiotic strains microencapsulated in a prebiotic matrix against SIF barrier, *in vitro*

Dependent variable: Microorganism growth (CFU·mL^{-1})			
Independent variables	**Categories**	**Mean**	***P* value**
Bile	Bile 0,1%	74.898^{a}	0.000
	Bile 0,2%	70.031^{b}	
	Bile 0,3%	64.117^{c}	
Formulation	Free of microorganisms	72.978^{b}	0.000
	Formulation 1	85.060^{a}	
	Formulation 2	67.426^{bc}	
	Formulation 3	56.441^{c}	
	Formulation 4	66.503^{d}	
Type of strain	*Lactobacillus*	71.054^{a}	0.055
	S. boulardii	68.310^{a}	

Averages that do not share a letter are significantly different

between *Lactobacillus* and *S. boulardii* yeast, demonstrating that the probiotic strains used have the capacity to tolerate bile salts, being able to develop their metabolic activities without being completely inhibited during the 4 h of exposure to SIF, *in vitro*, results that coincide with research that have evaluated the capacity of tolerance to bile of different strains [17, 18].

The results were similar to those reported by other authors who found a relatively high tolerance of *S. boulardii* to temperature, acid pH and bile salts up to 0.3% (w/w) using whey protein as encapsulation material, adding $CaCl_2$ or gum Arabic as optimal wall material, achieving high viability of *S. boulardii*, showing that microcapsules produced at higher drying temperature (125°C) showed higher resistance to gastric solution compared to lower drying temperature (80°C) with low resistance to the solution of gastric juice, demonstrating that the combination of different encapsulant materials enhances the protective capacity of microcapsules improving viability against simulated gastric conditions. Furthermore, viability decreased with increasing exposure to SIF, *in vitro* [23, 24, 25, 30].

In the same sense, the results of this research suggested that the combination of prebiotic polymeric materials (sodium alginate, oat flour and native cassava starch) of formulation 1, improves the survival of the mixed culture of probiotic strains against *in vitro* gastrointestinal simulation conditions, maintaining the average survival between 79.79 and 85.060%, demonstrating that this mixture of encapsulating material improves its protective barrier properties, results similar to those of other authors that suggest greater stability of the microcapsules produced with prebiotics than those that used only other types of material, showing higher initial counts, with respect to free bacteria [20, 26, 27, 28].

Viability of three strains of microencapsulated probiotic microorganisms in a prebiotic matrix with and without a carbon source

TABLE III show the results of the prebiotic test carried out on the four selected formulations, using controls with and without a carbon source to verify the prebiotic capacity of the encapsulating matrix. It was observed that there were significant statistical differences between the average counts of the four formulations and the controls with and without carbon source with $P<0.05$, with formulation 1 having the highest average count (9.0958 log CFU·g^{-1}) in relation to formulations 2, 3, and 4 and the controls (with and without carbohydrates), being this growth lower in the mixture of free strains without carbohydrates (6.6758 log CFU·mL^{-1}) compared to those exposed to MRS media with carbon source (sucrose), which may be related to the use of the carbohydrates present in the prebiotic microcapsules as a carbon source in the four formulations evaluated by the three strains of encapsulated microorganisms.

TABLE III
Viability of mixture of probiotic microorganisms with and without carbon source

Dependent variable: Microorganism growth (CFU·mL^{-1})			
Independent variables	**Categories**	**Mean**	***P* value**
pH	12	9.93228[a]	0.002
	0	9.42142[a]	
	8	8.94561[ab]	
	4	8.83226[ab]	
	16	8.58331[ab]	
	20	7.91603[ab]	
	24	6.54270[b]	
Formulation	with carbohydrates	11.8969[a]	0.000
	Formulation 1	9.0958[b]	
	Formulation 2	8.1944[bc]	
	Formulation 3	8.1908[bc]	
	Formulation 4	7.5238[bc]	
	without carbohydrates	6.6758[c]	
Type of strain	*L. rhamnosus*	9.664[ab]	0.000
	L. plantarum	9.129[a]	
	S. boulardii	6.994[b]	

Averages that do not share a letter are significantly different

The above is related to the exposure time, where statistically significant differences with $P<0.05$ are observed from time 0 to 24 h, with a decrease in counts as the exposure time to MRS broth with and without carbon source increases due to the depletion of the carbon source. Similar behavior has been reported by other authors, who indicated that the encapsulated microorganisms use the wall material as an energy source, maintaining their viability during exposure [3, 4, 19, 25, 29, 32].

It should be added that there were statistically significant differences with $P<0.05$, between the mean counts of *Lactobacillus* (9.664 and 9.129 log CFU·mL^{-1}) and *S. boulardii* yeast (6.994 log CFU·mL^{-1}) of the four formulations *boulardii* (6.994 log CFU·mL^{-1}) of the four formulations, where yeast activity decreased with increasing exposure time, showing that yeast has a more demanding carbohydrate requirement to reproduce and survive than *Lactobacillus*, since yeasts do not have a metabolic system to fragment polysaccharides. [4, 9, 32], needing the availability of sugars for growth; Another reason to explain this dilemma is the proportion of lactic acid bacteria, LAB, which produce acids derived from metabolism (fermentation), inducing acidic conditions necessary for their preservation by using the carbon source that served as a substrate for the mixed culture of probiotic strains, while yeast may be unable to change the acidic conditions in such a short time.

Many authors indicate that the consequence of the antagonistic effect, derived from the metabolites accumulated in the medium, are produced by the fermenting bacteria, especially acids such as propionate, acetate or lactate and their negative impact on the growth rate of yeasts, all of which would indicate that when LAB concentrations increase and these release greater quantities of substances into the medium, a phase of cellular quiescence or temporary halt to the multiplication of other organisms such as yeasts will occur [9, 33, 34].

FIG. 3 shows that the acidity production increased with increasing exposure time (24 h) of the microcapsules in MRS broth without carbon source (negative control), corroborating that the three materials (sodium alginate, oat flour (β–glycan) and native cassava starch) of the microcapsules, were used as carbon source in the four formulations by the mixed culture of microencapsulated probiotic strains, finding that there are no statistically significant differences with $P>0.05$ between the means of the acidity percentage values of the four formulations.

While the control samples (with and without carbohydrate source (sucrose) presented significant differences with $P<0.05$, indicating that the production of lactic acid and the reduction of pH values, as well as the simultaneous depletion of carbon sources as a reduction of growth microbial, they explain that LAB and yeast used the prebiotic material the microcapsules wall (starches, fibers (β–glycan)) as a substrate to produce acids derived from metabolism (fermentation), inducing acidic conditions necessary for their preservation and maintaining their viability during the exposure time (24 h), behavior similar to that reported by other authors who found that encapsulated probiotic microorganisms use the wall material as an energy source, preserving their viability during the exposure time [3, 19, 32].

It should be emphasized that recent studies have shown that β–glycan from oats possesses great prebiotic potential that cannot be digested by human digestive enzymes or absorbed in the upper intestinal tract, while possessing the ability to provide a carbon source to the intestinal microbiota within the distal intestinal tract region [15, 16], therefore it can be deduced that the use of oat flour in combination with other prebiotic materials enhances their properties, offering a feasible alternative to maintain the viability of a mixed culture of probiotic strains.

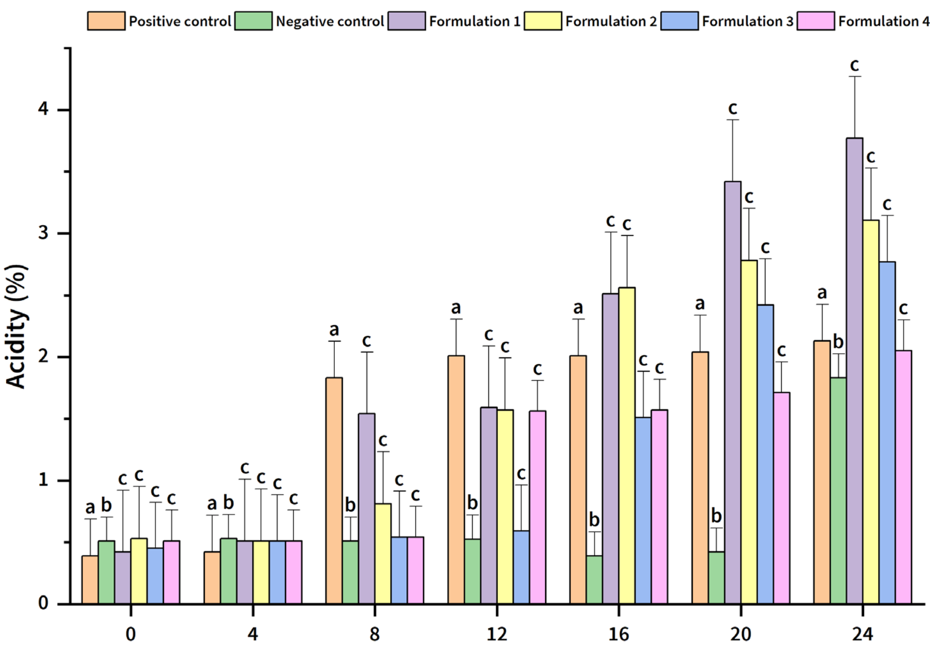

FIGURE 3. Percentage of acidity produced in MRS broth with and without carbon source (sucrose), *in vitro*

CONCLUSIONS

The mixture of polymeric material composed of sodium alginate, oat flour (β-glycan) and native cassava starch, exerts a protective barrier in gastrointestinal conditions *in vitro*, being an innovative alternative to improve the survival of a mixture of strains of probiotic microorganisms microencapsulated by spray drying, maintaining therapeutic levels (≥10^6 CFU·g^{-1}).

The best protective effect in the prebiotic microcapsules was in formulation 1 composed of sodium alginate (0.49%), native cassava starch (9.38%), oat flour (β-glycan) (2.13%), showing a higher survival rate of the mixed culture of microencapsulated probiotic strains in the *in vitro* gastrointestinal and prebiotic barrier conditions.

The prebiotic microcapsule wall material in all four formulations, composed of sodium alginate, oat flour (β-glycan) and native cassava starch serve as a carbon source maintaining the viability of a mixed culture of microencapsulated probiotic strains.

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Conflicts of interest

All the authors state that there is no significant financial conflict of interest or any other type of interest that could arise in the results or interpretation of this article.

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