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Physicochemical characteristics and survivability of *Lactobacillus paracasei* encapsulated by a gum arabic-pectin mixture as wall material and added to fresh panela cheese

Características fisicoquímicas y supervivencia de *Lactobacillus paracasei* encapsulado mediante una mezcla de goma arábiga-pectina como material de pared y adicionado a queso panela fresco

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Abstract

The aim of this research was to obtain and characterize microcapsules of *Lactobacillus paracasei* LBC81 LYO 10D (Lp-LBC) obtained by spray-drying using gum arabic-pectin mixture as wall material, evaluating their protective capacity and impact on sensory profile and acceptance of panela cheese used as food matrix. Microcapsules obtained at 170 °C showed the highest viability and adequate physicochemical and flow properties. Microencapsulated Lp-LBC showed higher viability during passage through the simulated gastrointestinal tract conditions compared to free cells. Also, microencapsulated bacterial cells showed greater viability than free cells in a fresh panela cheese, however some physicochemical properties changed during storage. The microcapsules showed a range size distribution from 5 to 150 μm which causing adverse changes in the sensorial properties of panela cheese. This study provides evidence of the protective capacity of a gum arabic-pectin mixture on the viability of Lp-LBC during spray-drying and through simulated gastrointestinal conditions, however, the particle size affects the physicochemical and sensory properties of the food in which said capsules were added.

Keywords: arabic gum, pectin, panela cheese, spray-drying, gastrointestinal simulation.

Resumen

El objetivo de esta investigación fue obtener y caracterizar microcapsulas de *Lactobacillus paracasei* LBC81 LYO 10D (Lp-LBC) obtenidas mediante secado por aspersión usando como material de pared una mezcla de goma arábiga-pectina, evaluando su capacidad protectora y su impacto en el perfil sensorial y de aceptación de queso panela usado como matriz alimentaria. Las microcápsulas obtenidas a 170 ºC mostraron la mayor viabilidad y propiedades fisicoquímicas y de flujo adecuadas. El Lp-LBC microencapsulado mostró una mayor viabilidad durante su paso por las condiciones gastrointestinales simuladas, en comparación con células no encapsuladas. Además, las células bacterianas microencapsuladas mostraron una mayor viabilidad que las células no encapsuladas en el queso panela fresco, sin embargo algunas propiedades fisicoquímicas cambiaron durante el almacenamiento. Las microcápsulas mostraron un rango de distribución de tamaño de 5 a 150 μm lo que generó cambios adversos en las propiedades sensoriales del queso panela. Este estudio proporciona evidencia de la capacidad protectora de la mezcla de goma arábica-pectina sobre la viabilidad de Lp-LBC durante el secado por aspersión y a través de las condiciones gastrointestinales simuladas, sin embargo, el tamaño de partícula afecta las propiedades fisicoquímicas y sensoriales del alimento en el cual dichas capsulas fueron adicionadas.

Palabras clave: goma arabiga, pectina, queso panela, secado por aspersión, sumilación gastrointestinal.

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1 Introduction

Lactobacillus paracasei is a Gram-positive lactic acid bacterium commonly found in fermented food and in the human intestinal tract, and its incorporation in foodstuffs has been proposed since different strains of this specie have shown probiotic properties such as modulation of the immune system and intestinal microbiota in adults, as well as increases the speed of response to influenza vaccine (Trachootham *et al*., 2017; Tao *et al*., 2019). In vitro studies have demonstrated that pure cultures of the probiotic strain *L. paracasei* subsp. *paracasei* NTU 101, exert antioxidant activities; while in in vivo studies the prevention of hypercholesterolemia, hyperlipidemia-induced oxidative stress and atherosclerosis have been reported in hamsters with a high-fat diet and administered with the fermentation products of this same probiotic strain (Tsai *et al*., 2009; Kwaw *et al*., 2018; Mantzourani *et al*., 2018). In parallel, some strains of *L. paracasei* are widely used in functional foods and healthy products in many countries (Toh *et al*., 2013). Regarding this, the scientific research has focused on the greater capacity of *L. paracasei* probiotic strains, which have high tolerance to acidic conditions compared to other beneficial microorganisms (Mantzourani *et al*., 2018). Based on the above, the ingestion of *L. paracasei* represents several advantages for human beings when is consumed in the recommended concentrations of viable cells ($\sim 10^6 - 10^7$ CFU/g) (FAO/WHO, 2002).

To achieve probiotic effect, the fundamental challenge is to assure the viability of the beneficial bacteria during its storge within the food matrix and at the passage through the gastrointestinal tract conditions, to increase its colonization and growth rate in gut (Ceja-Medina *et al*., 2021; Martínez-Preciado *et al*., 2021). In this sense, different technologies have been developed to protect the viability of microorganisms against the stressful conditions, improving their controlled delivery within foods and ensuring their positive bioactivities (Alfaro-Galarza *et al*., 2020). One of the methods to protect probiotics is microencapsulation, which allows to obtain microcapsules that can be stored either by freeze-drying, fluidized bed drying and spray-drying (Avila-Reyes *et al*., 2014; Arepally *et al*., 2020a; Juárez-Trujillo *et al*., 2021). The spray-drying is a widely used method to obtain stable bacterial microcapsules, due to its short exposure time to heat and fast particle drying, suitable for drying a variety of heat-sensitive products with relative simplicity, low cost for bulk production and high efficiency (Ceja-Medina *et al*., 202x1; Martínez-Preciado *et al*., 2021).

There are different types of wall materials that can be used as encapsulating agents in spray-drying process. In addition to protect the viability of encapsulated microorganisms through the gastrointestinal system, wall materials should possess emulsifying and film-forming properties, must be biodegradable, show low viscosity at high solid contents, exhibit low hygroscopicity and must be low cost (Fazilah *et al*., 2019). Several encapsulating agents such as hydrocolloids and prebiotics have been used to protect probiotics from thermal damage and dehydration during spray-drying (Avila-Reyes *et al*., 2014; Cortés *et al*., 2014).

Prebiotics are non-digestible food components that beneficially affects the host health, since they selectively stimulate the growth and proliferation of probiotic microorganisms in colon to provide health benefits such as pathogens inhibition, immune system stimulation, reduction of blood lipid levels and improve of bioavailability of minerals (Azam *et al*., 2020). Also, the integration of prebiotics in the encapsulation process provides greater survivability rate of encapsulated bacteria during processing and storage (Paim *et al*., 2016; Ceja-Medina *et al*., 2021). Most lactic acid bacteria can ferment fructo-, galacto- and hetero-oligosaccharides used both as prebiotics and wall materials in encapsulation process (Ceja-Medina *et al*., 2021). Water-soluble fiber, native starch and inulin have been some of the prebiotics used as wall materials for the encapsulation of lactic acid bacteria with probiotic properties. Peredo *et al*. (2016), reported the use of prebiotic *Plantago psyllium* as wall material in the encapsulation of three *Lactobacillus* species. The findings revealed a higher encapsulation efficiency and better protection of bacteria viability under storage and gastrointestinal conditions, in comparison to capsules made of prebiotics inulin and potato starch as wall materials.

Another material used to protect probiotics under technological and physiological stress conditions is gum arabic. This material shows excellent emulsifying properties and low viscosity, at low levels (1-10%) acts as film former, moisture stabiliser and mouth feel enhancer (de Matos-Jr *et al*., 2019). Furthermore, gum arabic has shown prebiotic properties as it can be selectively fermented by lactobacilli and bifidobacteria into short chain fatty acids (Leylak *et al*., 2021). As wall material,

gum arabic can establish electrostatic interactions with other polymers to improve the protection of encapsulated microorganisms. Eratte *et al*. (2015), encapsulated the probiotic Lactobacillus casei 431 in a single whey protein isolated-gum arabic complex coacervate, which showed electrostatic compatibility and less loss of viability after spray-drying. Recent studies have increasingly focused on the use of gum arabic and spray-drying to encapsulate probiotics. Nale *et al*. (2018), encapsulated the probiotic consortia from kefir, while Fazilah *et al*. (2019), encapsulated the probiotic bacteria Lactococcus lactis Gh1. Both studies agree that the use of gum arabic as wall material and the subsequent spray-drying generates better protection to the viability of encapsulated microorganisms and provide stability to the powdered product, facilitating its addition to food matrices without affecting their sensorial attributes. Alternatively, biopolymers mixtures, including gum arabic and other hydrocolloids such as pectin, have been used to increase the efficiency of encapsulation of probiotic microorganisms for their subsequent addition within food matrices, thus generating new functional foods (Leylak *et al*. (2020).

As mentioned above, pectin is a negatively charged hydrocolloid used as wall material in the microencapsulation, because its gelation properties facilitate emulsion elaboration and generate stable capsules, after spray-drying. In addition, pectin shows prebiotic characteristics since, as it is not digested by human enzymes and thus stimulates the growth of beneficial bacteria within intestinal microbiota (Dafe *et al*., 2017; Sun *et al*., 2020). Based on these findings, the use of gum arabic and pectin as wall materials with electrostatic stability and prebiotic behaviour, can increase the viability of encapsulated probiotics exposed to processing, storage, and digestive conditions when they are included in food matrices.

According to the above, consumers are increasingly demanding products fortified with probiotic bacteria (McKenzie *et al*., 2018). The application of probiotic cultures in different food matrices could represent a great challenge (Shori, 2016), not only due to the generation of new functionals foods, but also due to the modification of well-accepted food products such as cheeses, to offer popular food products enriched with live probiotic culture with benefits to the consumers. Most Mexican cheeses are fresh and represent about 80% of the consumed cheeses (Jiménez-Guzmán *et al*., 2009). Among them, panela cheese is one of the most consumed fresh cheeses in Mexico, recognized for its sensory and nutritional attributes, high moisture and soft structure, which can facilitate its use as food matrix for the inclusion of encapsulated probiotic bacteria such (Ramírez-López & Vélez-Ruiz, 2018; Carrillo-Lopez *et al*., 2020). In previous studies, fresh cheeses such as panela, have been chosen as a model food to convey nutraceuticals to consumers, since these products are much-appreciated and well accepted foods within population (Pereira *et al*., 2017). However, there are few studies focused on the impact generated by the addition of encapsulated microorganisms on the sensory properties of the fresh cheeses used as food matrix. Based on this, the selection of the wall material and encapsulation method are the key factors to obtain probiotic capsules with specific physicochemical characteristics that promote their addition to food matrix without affecting its stability and sensorial profile, as well as guarantee the protection and correct liberation of the encapsulated microorganisms. Hence, the aim of this study was to obtain and characterize the spray-dried microcapsules of *L. paracasei* LBC81 LYO 10D produced with a gum arabic-pectin mixture as wall material, evaluating their protective capacity and their impact on sensory profile and acceptance of a panela cheese used as food matrix.

2 Material and methods

2.1 Bacterial growth conditions and inoculum standardization

Lyophilized (1 g) *Lactobacillus paracasei* strain LBC81 LYO 10D (Lp-LBC) (Danisco, France), was inoculated and grown into 200 mL of de Man Rogosa and Sharpe (MRS) broth at 37 ºC until reaching the end of the exponential growth phase, monitoring its growth by turbidimetry at 600 nm in a spectrophotometer UV-vis (Jenway, mod. 6305, UK). Later, the bacterial suspension was harvested by centrifugation at 2935 x g for 10 min at 4 ºC and washed twice with a phosphate buffer solution (PBS, pH= 7.0). The washed bacterial cells were resuspended in water:glycerol solution (50:50 v/v) and kept at -80 ºC until use.

For the preparation of inoculum, the frozen Lp-LBC was inoculated at 1% (v/v) in 100 mL of MRS broth and incubated at 37 ºC for 18 h (time required to reach the end of exponential growth). After this time,

the bacterial cells were harvested by centrifugation, the supernatant discarded and the pellet washed with PBS under the same conditions. Then, the bacterial pellet with viable cells concentration of 6×10^{11} CFU/g was used as inoculum for elaboration of bacterial emulsion.

2.2 Preparation and characterization of bacterial emulsion

For the preparation of bacterial emulsion, the inoculum of Lp-LBC (washed bacterial pellet) previously obtained, 3 g of high methoxyl pectin (previously hydrated 24 h before use at 25 ºC, Conquimex, Mexico), 20 g of gum arabic (Cosmopolitan Drug Store, Mexico) and 77 g of sterile distilled water were mixed and homogenized at 13000 rpm for 5 min using an immersion homogenizer (Wiggen-Hauser, mod. D-500, Germany). Once the homogenization time was over, the prepared bacterial emulsion (100 g), with a of high methoxyl pectin:gum arabic ratio of 3:20 (1:7.66 w/w), was characterized as detailed below.

Firstly, the pH of emulsion was measured at 25 ºC using a potentiometer, following the recommendation from the manufacturer. Later, the surface tension was measured by Wilhelmy plate method, using a tensiometer (DataPhysics, model DCAT11, Germany), equipped with a platinum-iridium plate (dimensions: 10 mm long, 19.9 mm wide and 0.2 mm thick), with a speed of 5 Hz and an immersion depth of 3 mm at 25±1 °C (Juárez-Trujillo *et al*., 2021).

The viscosity of the bacterial emulsion was evaluated using an analog viscometer (Brookfield mod. RVT, USA) at different speeds (from 0.5 to 100 rpm). The viscosity was calculated according to the following equation (Eq. 1) and expressed in centipoises (cP). "Viscosity = (L x F)" Eq. 1 Where L is the value recorded at each given speed (rpm) and F is the conversion factor given for the specific needles used in the assay. The results were expressed as the average of the viscosity value of all speeds.

The particle size was determined by the laser diffraction method using a Coulter Counter (Beckman Coulter, mod. LS32, USA), equipped with a photodiodes array. The results were reported as the average of the size distributions of particles dispersed in the bacterial emulsion. Subsequently, both polydispersity index (PdI) and ζ-potential were evaluated in the emulsion diluted with deionized water (1:10 v/v) at 25 ºC, using a Zetasizer (Nano-Zs, Malvern Instruments, model ZEN 3600, UK).

2.3 Spray-drying of the bacterial emulsion

Once characterized, the bacterial emulsion was dried in a laboratory spray dryer (Büchi, mod. B-290, Switzerland) at three different inlet temperatures (150, 170 and 190 ºC), with an outlet temperature of 90 ºC, a feed rate of 4 g/mL, 60 mmHg of vacuum pressure and equipped with a 0.7 mm diameter spraying nozzle. Later, the dry microcapsules, collected in sterile glass bottles attached to the bottom of the cyclone, were stored at 5 °C until their viability analysis and characterization. Each drying process was carried out in triplicate.

2.4 Enumeration of viable Lp-LBC and encapsulated efficiency

1 g of spray-dried capsules at three different inlet temperatures (150, 170 and 190 ºC), was used to evaluate the viability of encapsulated Lp-LBC. Firstly, the dry capsules were dissolved in 9 mL of PBS, being stirred for 6 h at 25 ºC. After this time, serial decimal dilutions, using the same buffer, were made until obtaining a 1:109 CFU/g dilution. The viability of the released cells was quantified using the plate count method in MRS agar incubated 48 h at 37 ºC. The encapsulation efficiency was calculated according to the following equation:

$$\text{Encapsulation efficiency } (\%) = \frac{N}{N_0} \times 100 \qquad (1)$$

Where N and N_0 are Log CFU/g after and before encapsulation process and drying process, respectively.

2.5 Characterization of dry microcapsules

2.5.1 Physicochemical properties

The moisture content was determined gravimetrically in freshly spray-dried capsules and calculated according to the methodology of AOAC (1995), using a drying oven at 102±2 ºC, until constant weight was observed. The moisture content was expressed as percentage (%) given in dry basis (db). The water activity was measured at 25 ºC using equipment AquaLab 4 TE (Decagon, USA). The hygroscopicity was determined according to the procedure described by Fritzen-Freire *et al*. (2012), with slight modifications. One gram of dry microcapsules was placed in an airtight glass desiccator containing NaCl saturated solution of 75 % relative humidity and stored for a week at 25 ºC. At the

end of storage time, the sample was weighed, and then hygroscopicity was calculated using the following equation (Eq. 2) (Arepally *et al*., 2020a):

$$\text{Hygroscopicity}\left(\frac{gH_2O}{100g\,sample}\right) = \frac{m_f - m_i)}{m_i} \times 100 \quad (2)$$

Where m_f and m_i are the weight of sample before and after the storage period, respectively.

2.5.2 Bulk and particle density

Particle density, bulk density and tapped bulk density were determined immediately after drying process according to the methodology proposed by Islam *et al*., (2017). The particle density was measured by a pycnometer method using toluene as solvent, and then dividing the total mass of the particle by its total volume. The bulk density was determined by pouring 2 g of dry microcapsules, which were loosely weighed into an empty 10 mL graduated cylinder. Subsequently, the cylinder with the sample was slightly tapped until constant volume, considered as tapped bulk density.

2.5.3 Flow properties

The compressibility and Hausner ratio (HR) were analyzed according to the methodology followed by Arepally and Goswami, (2019); to evaluate the flowability and cohesiveness of dry microcapsules, respectively. To calculate the compressibility and HR, the equations (Eq. 3 and 4), given by Reddy *et al*. (2014) were used.

$$\text{Compressibility (\%)} = \frac{\text{Tapped bulk density}\left(\frac{g}{cm^3}\right) - \text{Bulk density}\left(\frac{g}{cm^3}\right)}{\text{Tapped bulk density}\left(\frac{g}{cm^3}\right)} \times 100 \quad (3)$$

$$\text{Hausner ratio} = \frac{\text{Tapped bulk density}\left(\frac{g}{cm^3}\right)}{\text{Bulk density}\left(\frac{g}{cm^3}\right)} \quad (4)$$

2.5.4 Color analysis

A ColorFlex (HunterLab, mod. V1-72, USA) colorimeter was used to measure the CIE Lab color L^* (lightness to darkness), a^* (greenness to redness) and b^* (yellowness to blueness), parameters of the dry microcapsules. In addition, color hue angle (ºHue) and chromaticity (Chroma) were calculated using the a^* and b^* parameters, according to the equations 5 and 6, respectively.

$$^{o}Hue = \arctan(b^*/a^*) \quad (5)$$

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (6)$$

2.6 Effect of pH on viability of encapsulated Lp-LBC

Non-microencapsulated bacteria and dry microcapsules were separately incubated at 37 ºC in 9 mL of MRS broth adjusted to pH values of 1.0, 2.0, 3.0, 5.0 and 6.0 with 12 N HCl and 1 N NaOH solutions. Samples incubated in MRS broth with no pH adjust were used as control (pH 6.5). After 0.5, 1.0, 1.5 and 6 h exposure, samples were taken, and then bacteria viable count was obtained by the plate count method in MRS agar after 48 h at 37 ºC. For the enumeration of viable encapsulated bacteria, the microcapsules previously exposed to the different pH values were dissolved in PBS and the released viable microorganisms were enumerated under the same conditions in MRS agar. All the experiments were performed in triplicate.

2.7 Survival of free and encapsulated bacteria under simulated gastrointestinal conditions

Survival to gastrointestinal conditions were determined according to the methodology proposed by Ortakci and Sert (2012), with slight modifications. The samples of dry microcapsules (5 g) and free bacteria cells suspension (5 g), both with a similar viable cells concentration (10^9 CFU/g), were separately added to 45 mL of an artificial gastric fluid consisting of 3 mg/mL pepsin (Sigma, St. Louis, USA), dissolved in a sterile electrolyte solution of NaCl 0.85% (w/v) at pH 2.0. After, 30, 60 and 90 min of incubation at 37 ºC in gastric fluid, 1 mL aliquots of both samples were removed, serially diluted with PBS and spread-plated onto MRS agar. Subsequently, the remaining gastric fluids were transferred and mixed with an artificial intestinal bile juice, prepared with 1.2% bile salts (w/v) (Oxgall, Sigma, USA) and 1.9 mg/mL pancreatin (Sigma, St. Louis, USA), dissolved in MRS broth (pH 6.5) at 37 ºC and kept under constant agitation during 360 min. After this time, one-milliliter aliquots were again removed, serially diluted with PBS and spread-plated onto MRS agar, thus determining the survival capacity of the encapsulated and free

bacteria expressed as number of viable cells (Log CFU/g).

2.8 Morphological analysis of the microcapsules spray-dried

Prior to the observation with a scanning electron microscope (SEM) (Jeol, mod. JSM-5600lv, Japan), the freshly spray-dried microcapsules (1 g), were placed inside a glass desiccator previously equilibrated to a moisture content of *ca*. 0.00, using phosphorus pentoxide as desiccant agent. The spray-dried capsules were kept this condition during 20 days at 25 ºC. After this time, the dehydrated microcapsules were removed from the desiccator and coated for 45 sec with a mixture of gold-palladium (60:40), and then observed with the SEM at 28 kV. The micrographs obtained at different magnifications were used to evaluate the morphological parameters of area, perimeter and Feret's diameter of microcapsules in the software ImageJ v. 7.50i (National Institute of Health, USA).

2.9 Incorporation of free and encapsulated cells into fresh panela cheese

Cow raw milk was pasteurized at 74 °C for 30 s. After cooling to 34°C, it was added with 40 g/L of powdered milk, 9 g/L of sodium chloride, 0.7 mg/L of calcium chloride and 0.035 g/L of animal rennet (consisting roughly of chymosin and pepsin). The mixture was rested at 33 °C for 40 min to favor the curd formation. The curd obtained was then cut to start the whey release process, being drained after 5 min rests, and kneaded to remove the remaining whey. Once the drained curd was obtained, the free and encapsulated Lp-LBC cells were added to reach a final concentration of 9 Log CFU/g. The cheeses (100 g pieces) were added with 10 g of Lp-LBC capsules and left to rest for 16 h at 8 ºC, to later evaluate their viability, moisture content and total color change (ΔE) at 0, 7, 14 and 21 days of storage. The results obtained were compared with those of panela cheeses added with non-encapsulated Lp-LBC cells, stored under the same conditions.

2.10 Sensory evaluation

A total of 100 untrained judges with an age range between 20-40 years, participated in a sensory evaluation of a fresh panela cheeses (at 0 days of storage), added with free and encapsulated cells of Lp-LBC, using a fresh panela cheese without microorganisms as control. The judges were selected using a predetermined screening criteria based on purchasing and consumption frequency as well as familiarity with the typical dairy products. Consumers were included and considered in the analysis when they reported consuming dairy products at least 1-3 times a week and agree to participate. Samples were labeled with randomly generated three-digit numbers. After reading the consent form, judges were asked to indicate their preferences on flavor, texture, color, gritty and general preference of the cheese, using a 15 cm unstructured scale with the labels "Agree" and "Disagree" to the ends of the line. Data were translated to cm for further analysis. Samples were presented in white plastic cups and unsalted crackers were used as a nearly flavorless carrier for the sample. Product temperature was carefully controlled, and presentation of all samples was uniform.

2.11 Statistical analysis

For the statistical analysis, the statistical package Minitab 16 (Minitab Inc. State College, USA) was used, and a multivariate factorial ANOVA was used for the comparisons. Averages of triplicates were analyzed using the Tukey test with significance level (α) of 0.05. For the statistical analysis of the sensory evaluation, the non-parametric Mann-Whitney U test was used.

3 Results and conclusions

3.1 Characterization of bacterial emulsion

The values obtained for the determinations of pH, surface tension, viscosity, particle size, ζ-potential and PdI of bacterial emulsion are shown in Table 1. Specifically, for the analysis of particle size, the equipment used allowed to identify two peaks of size abundance, which is why the values of particle size range between 0.450 and 2000 μm. The presence of this range in particle size is mainly due to the combination of the two wall materials, which have a significant influence on the droplet size in the emulsion (Silva *et al*., 2014). This effect can be explained by the strong repulsive forces between the formed droplets, mainly due to the combination of electrostatic and steric forces derived from the proteinaceous content of gum arabic positively charged and high methoxyl pectin

Table 1. Physicochemical properties of *L. paracasei* LBC LYO 10D emulsion.

Properties	Value
pH	4.67±0.02
Surface tension (mN m^{-1})	52.47±0.03
Viscosity (cP)	120.00±5.10
Particle size (μm)	0.470-2000 (peak 1:0.470-60) (peak 2:61-2000)
ζ-potential (mV)	-20.13±2.31
PdI*	0.58±0.11

(a negatively charged polysaccharide) (Esfanjani *et al*., 2017; Sun *et al*., 2020; Sabet *et al*., 2021). It has been reported that the use of pectin produces strong gels with high encapsulation efficiency and stability, related to the charge and methoxylation degree of the pectin molecule (Lee *et al*., 2019; Sun & Wicker, 2021). The gelation capacity of pectin first generates an increase in emulsion viscosity and later a larger size in the droplets obtained from the stirring (Hartini *et al*., 2021). Furthermore, esterified groups of high methoxyl pectin can interact with the H-1 and H-2 groups of glucoronic acid units of alginate, generating layers on the surface of alginate capsules leading to lower porosity, larger particle size and a decreased in surface-volume ratio resulting in less diffusion of the active center, the latter derived from the formation of membranes with high resistance to rupture and coalescence (Esfanjani *et al*., 2017; Arab *et al*., 2019). Previous studies have shown that the increase in droplet size within the emulsion is related to the concentration of encapsulating polymers and the stirring speed (Valero-Cases & Frutos, 2015; Azam *et al*., 2020). Regarding stirring speeds, it has been reported that a stirring speed of 12,000 rpm, generates an emulsion with a droplet size of 0.42 μm, when alginate is used as encapsulating agent. However, the addition of different polymers such as high methoxyl pectin, increased the particle size up to 141 μm (Arab *et al*., 2019). This change in size is mainly due to the increase in viscosity caused by the addition of pectin, which directly affects the droplets size and even the size of the capsules once they are spray dried (Hartini *et al*., 2021). In line with these findings, the stirring conditions used in this study could generate a particle size similar to that reported, however, the addition of pectin significantly increased the particle size within the emulsion, thus possibly a redesign of formulation can be implemented to facilitate particle formation within different encapsulation methods such as spray drying. Particle size is one of the factors that limit the use of microcapsules in foods since they can interfere with the sensory attributes and acceptance of the product when the diameters of capsules exceed 100 μm. So, the largest capsules may be added in food matrices with similar characteristics such as viscosity, granular texture or gel-like structure, in order to increase their acceptance (Cardoso *et al*., 2020; Kavas *et al*., 2021).

ζ-potential is one of the commonly parameters used for emulsion stability studies. The values of ζ-potential refer to which of the two types of mechanisms is responsible for the stability of the emulsion. The steric mechanism comprises the adhesion of the emulsifier to the surface of droplets, and the electrostatic mechanism is associated with the electrostatic repulsion of the surface charges of the particles (Sena *et al*., 2019). Generally, the higher the absolute value of ζ-potential (>±30 mV), the stronger the electrostatic repulsions and the more stable the emulsion (Xiong *et al*., 2019). Conversely, lower ζ-potential values (<±30 mV), such as those observed in this study (-20 mV), may confirm the steric mechanism as dominant in the stability of the emulsion (Sena *et al*., 2019). This steric mechanism in the gum arabic gum/pectin, confirmed by the negative charge of ζ-potential may be possibly due to the carboxylic acid and anionic behaviour of gum arabic and pectin biopolymers due to carboxylic acid content in their structure, which gives them a negative charge (Timilsena *et al*., 2016). In the same way, negative charged ζ-potentials have been observed in emulsions with high concentrations of gums (such as gum arabic), in which, a high absolute ζ-potential value was related to stable systems (Mirhosseini *et al*., 2008). Such stability is closely related to the electric charge of emulsion droplets surface, since a strong repellent force among droplets stabilize the emulsion (Shamsara *et al*., 2017). In this case, the negative ζ-potential is induced by the molecular cohesive forces between gum arabic and pectin, which provide a mechanical barrier on the surface of the dispersion and a repulsion force that can contribute to the stabilization of the bacterial emulsion.

The pH is an important factor to maintain emulsion stability since its variation may indicate chemical changes and a subsequent emulsion destabilization. Generally, emulsions have pH values between 2.5-7.5 and any change outside these values is considered an effect of system instability (Sena *et al*., 2019). Other key factor to consider in emulsion stability

analysis is the surface tension. The generation of small droplets trough high energy homogenization processes increases the surface tension due to presence of frictional forces between the droplets caused by the large surface area. Conversely, a decrease of surface tension may induce the accumulation of emulsion droplets at the air/emulsion interface (cream) (Yang *et al*., 2018; Sena *et al*., 2019). The surface tension of pure water at 25 ºC is 72.4 mN m^{-1} and compared to this, the emulsifying agents such as pectin contribute to decrease this value to obtain a more stable emulsion. However, it has been reported that pH variations are also related to the increase or decrease in surface tension of emulsions made with pectin (Yang *et al*., 2018). Likewise, the arabic gum has shown a significant effect on the surface tension of emulsions mainly due to its ability of lowering surface tension between water and oil, forming a more stable emulsion. So, a good emulsifier should be able to bind easily at the interface created during homogenization, reducing surface tension to facilitate droplet breakage (Silva *et al*., 2015; Dara, 2021). Based on this, the values of pH and surface tension reported in this study confirm the elaboration of a emulsion made of gum arabic/pectin with desirable stability characteristics in terms of pH-dependent surface tension (Dara, 2021).

Emulsion properties are crucial to obtain a successfully microencapsulation process. In addition to droplet size, viscosity of emulsion and PdI are important properties to considerer before spray drying encapsulation. It has been noted that pectin increases both the viscosity of the emulsion and internal resistance of droplets, which affect the outcome of the atomization process by narrowing the particle size distribution (Hartini *et al*., 2021). Secondly, the PdI represents the distribution of uniform particles in the sample, the lower the PdI value, the more uniform the particle size (Strobel *et al*., 2016). The emulsion prepared with gum arabic/high methoxyl pectin exhibited a low viscosity value of 120 cP, which agrees with that reported by Hartini *et al*. (2021), for an emulsion prepared with jelly fig pectin with a viscosity range from 17 to 186 cP. This low viscosity could generally produce small size droplets during spray drying, however, the PdI of 0.58 suggests the presence of a heterogenous distribution of particle size in emulsion and after spray drying.

3.2 Viability of encapsulated Lp-LBC and physicochemical properties of dry microcapsules

The microencapsulated Lp-LBC cells showed a viability of 9.15 Log CFU/g when they were spray-dried at an inlet temperature of 170 °C. This viability was significant higher ($p<0.05$) than those observed at inlet temperatures of 150 and 190 ºC.

Table 2. Viability of encapsulated *L. paracasei* LBC LYO 10D and physicochemical properties of capsules spray-dried at different temperatures.

Parameter	**150 °C**	**170 °C**	**190 °C**
Viability (Log CFU/g)	8.42±0.34a	9.15±0.14b	8.22±0.25a
Moisture (% db*)	7.70±0.31c	6.95±0.22b	2.88±0.31a
Water activity	0.355±0.27a	0.315±0.21a	0.269±0.31a
Hygroscopicity (g H_2O/100 g solids)	20.15±0.40c	17.50±0.35b	15.30±0.34a
Bulk density (kg/m^3)	320±0.05a	280±0.08a	290±0.06a
Tapped density (kg/m^3)	415±0.08a	360±0.09a	390±0.10a
Compressibility (%)	24.10±1.30a	22.50±2.90a	25.90±2.50a
Hausner ratio	1.31±0.20a	1.28±0.10a	1.34±0.09a
Particle density (kg/m^3)	780.30±5.30b	920.50±5.50c	670.32±7.10a
Color parameters			
*L**	70.07±0.34a	86.54±0.04c	79.68±0.25b
*a**	-0.983±0.11b	-0.46±0.12a	-0.51±0.15a
*b**	10.72±0.27a	11.57±0.05b	10.65±0.31a
ºHue	84.78±1.51a	87.72±0.08b	87.29±1.67b
Chroma	10.76±0.30a	11.57±0.05b	10.66±0.31a

*db: dry basis.

This significant increase of viability at 170 ºC inlet temperature can be attributed to the moisture content of dry capsules since it has been observed that a moisture content between 4 and 7% generates a greater stability of dry capsules and greater viability of the encapsulated microorganism (Arepally *et al*., 2020b). Based on this, the highest viability observed in the capsules spray-dried at 170 ºC inlet temperature can be explained as a function of their moisture content (6.95% db), ensuring high stability and protection of encapsulated probiotic. Likewise, the effect of moisture content on viability was confirmed by the capsules spray-dried at 150 and 190 ºC inlet temperatures, which showed less viability of encapsulated probiotic bacteria related to their moisture content of 7.70 and 2.88% db, respectively. Regarding water activity, the dry microcapsules showed values from 0.269 to 0.355 without significant differences ($p>0.05$) between samples. In the same way, the hygroscopicity of microcapsules was affected by the inlet temperatures, since at 150, 170 and 190 ºC, the values were of 20.15, 17.50 and 15.30 g H_2O/100 g solids, respectively.

Immediately after drying, the physicochemical properties of the microcapsules were evaluated. Encapsulation efficiency is one of the most important parameters affected by the encapsulation process and selection of the wall matrix (Çabuk & Tellioğlu, 2015). In this study, the inlet temperatures in spray-drying directly affect the encapsulation efficiency, since at 150 and 190 ºC the efficiency was of 77.53 and 69.83%, respectively. The increase of inlet temperature during spray-drying generates a thermal and dehydration inactivation of cells that begins with the denaturalization of bacterial components such as DNA, RNA, proteins, and ribosomes (Arepally *et al*., 2020a). However, at an inlet temperature of 170 ºC the encapsulation efficiency (77.74%) was higher than those showed above, this due to the presence of a moister content of 6.95% that improves the capsule stability and viability of the encapsulated microorganisms (Arepally *et al*., 2020b). Also, the moisture content and water activity of a powder are important parameters that influence stability during storage and operating parameters during drying (inlet/outlet temperatures and solids concentration). It has been reported that satisfactory encapsulation efficiency is obtained with a lower inlet temperature of 100 ºC, but when a higher outlet temperature of 80-90 ºC are used, this encapsulation efficiency is lesser in comparison to spray-dried microcapsules at 40-60 ºC as outlet temperatures (Fu & Chen, 2011). The outlet air temperatures influence the encapsulation efficiency; however, the capsules microstructure is determined by the inlet air temperatures (>100 ºC). These temperatures generate a strong physical barrier in capsules to properly protect and release the active center under controlled conditions. So, in the present work, the inlet temperatures (150, 170 and 190 ºC) were considered determining parameters to obtain the greatest protection of the bacteria encapsulated by spray drying (Martínez-Preciado *et al*., 2021). In contrast, the moisture content and water activity should be optimized to obtain a powder with improved stability and shelf-life; and an optimum viability of bacteria (Avila-Reyes *et al*., 2014). In this work, the higher viability (9.15 Log CFU/g), was observed in capsules dried at an inlet temperature of 170 ºC, with a final moisture and water activity of 6.50% and 0.315, respectively. These results are similar to that reported by Arepally *et al*. (2020a), who obtained a concentration of viable *Lactobacillus acidophilus* NCDC 016 cells between 7.3-9.97 Log CFU/g in spray-dried microcapsules with final moisture content of 4.59-9.05% and water activity of <0.6. According to the authors, these results indicate stability of the capsules against detrimental chemical and microbiological reactions and can improve the probiotic effects of bacteria.

In encapsulation by spray-drying, the moisture content, water activity and hygroscopicity are closely related to the polymer used as wall material. Polymers such as gum arabic can increase the encapsulation efficiency due to the formation of a proteinaceous protective layer on the microbial cell wall with some hydrophobic amino acids residues that can reduce the heat transfer in the spray-drying (Sanchez *et al*., 2018). Compared with the activity of water, the hygroscopicity of the microcapsules increased when they are dried at low inlet temperatures, this facilitates the retention of water from the environment, by the protein and carbohydrate residues present in gum arabic (Arepally & Goswami, 2019).

Regarding flow properties, bulk and tapped densities of the dry capsules did not show significant differences ($p>0.05$) between the spray-dried treatments. This same phenomenon was observed in the other properties that provide information about flow properties of the capsules, such as HR and compressibility, which ranged from 1.31 to 1.34 and 22.50 to 25.90, respectively. Similarly, particle density varied from 670.32 to 920.50 kg/m^3 being the capsules spray-dried at 170 °C those that presented the highest value, however, no significant differences

(p>0.05) with the values obtained at 150 and 190 ºC, were observed. The knowledge about physicochemical characteristics of dry capsules such as flow properties helps in the selection of the food matrix where they can be added, as well as the type of packaging to be used and the optimal storage conditions. Flow properties determine the quality of powdered foods, being used to determine their behavior during storage, processing and transport (Cancino-Castillo *et al*., 2020). Thus, flow properties are important quality parameters for the industrial production of dry microcapsules, including probiotic microcapsules such as those obtained in this study. Tapped density is an important property for determining the weight and amount of material that fits inside a container and how should be stored (de Barros Fernandes *et al*., 2014). Tapped density includes the interstitial volume and pore volume of the microcapsules. Both parameters indicated flowability of the microcapsules (Parthasarathi & Anandharamakrishnan, 2016). Other parameters affecting the flowability of microcapsules are related to the irregular flaky structures that increase the frictional force and to the angle of repose, which reveals the cohesiveness, particle size and surface area of the microcapsules. The particle density is a well-defined quantity which represents the true density of the particles that make up the powder, indicating that microcapsules dried at inlet temperature of 170 °C have fewer pores on their surface (Islam *et al*., 2017). It has been reported that both an increase in inlet air temperature as well as the gum arabic causes a reduction in bulk density. The gum arabic increases the viscosity emulsions and leads to production of large droplet size, whilst the inlet air temperatures produce faster evaporation rates and more porous structure on the capsule surface (Arepally *et al*., 2020a). These conditions affect the flowability, porosity and particle density of the microcapsules obtained, making the even hollow (Goula & Adamopoulos, 2010). The flowability of microcapsules are related to the water content in capsules surface. The water causes particles to stick together and thus, increase their resistance to flow (Arepally *et al*., 2020a). Based on the results obtained for HR and compressibility, the microcapsules obtained in this work have poor flowability and lower compressibility in comparison to microcapsules of *Bifidobacterium animalis* subsp. *lactis* (BLC-1) made of agave fructans, which showed a HR and compressibility of 1.48 and 32%, respectively, after being spray-dried with an inlet temperature of 160 ºC (Juárez-Trujillo *et al*. 2021).

According to color analysis, the L^* values of dry capsules were statistically different in all the treatments, while the b^* and Chroma were similar in capsules obtained with inlet temperatures of 150 and 190 ºC. Despite the difference with the capsules obtained with an inlet temperature of 150 ºC, the ºHue locates the color of the capsules of all treatments in the first quadrant of the color plane, with values close to 90º, and a chromaticity linked to the white-beige colors area. These results differ from those reported by Arepally & Goswami (2019), who observed that color parameters of the capsules of L. acidophilus made with gum arabic as wall material, especially L^*, were affected by the inlet temperatures and concentration of gum arabic. The decrease in L^* values of the capsules are related to the intensity of browning of the carbohydrates from gam arabic and can be identified as a lightness reduction in the capsule surface.

3.3 Effect of pH on viability of encapsulated Lp-LBC

Free Lp-LBC was very sensitive to low pH conditions. Table 3 shows that the viability of the free bacteria was more affected at pH=1, in which a complete loss of viability was observed after 0.5 h of incubation. In contrast, at this same pH value, the viability of encapsulated Lp-LBC decreased 4.72, 4.95 and 5.56 logarithmic cycles (Logs) at 0.5, 1.0 and 1.5 h of incubation, respectively. At pH 6.5, encapsulated Lp-LBC exhibited a decrease about of 1.21 Logs after 1.5 h incubation, while free Lp-LBC showed a decrease of only 2 Logs, after the same incubation time. These results can be explicated because pH of 5-6 is the optimum for Lp-LBC growth, which affects its viability to a lesser extent. One of the challenges of encapsulated probiotics is that they resist the mechanical properties and the variety of pH conditions of the foods in which they are incorporated. Viability losses during processing, storage and gastrointestinal transit reduce the functionality of probiotics to exert health benefits. On the other hand, viability of free and microencapsulated microorganisms was mostly affected when the pH was lower. Low specific pH affects the interactions between the two wall materials, since when the pH value is well below the isoelectric point of both biopolymers, they have opposite surface charges that cause the formation of strong and complex particles with larger sizes, which finally formed a relatively large precipitating aggregates denominated flocs.

Table 3. Effect of pH on viability of microencapsulated *L. paracasei* LBC LYO 10D.

pH value	Sample	Viable cell count (Log CFU/g)			
		0 h	0.5 h	1 h	1.5 h
1	Free	9.15±0.14	ND*	ND	ND
	Encapsulated	9.15±0.14	4.43±0.34	4.20±0.18	3.50±0.15
2	Free	9.15±0.14	4.52±0.41	4.40±0.42	4.10±0.10
	Encapsulated	9.15±0.14	7.50±0.21	7.32±0.28	6.46±0.29
3	Free	9.15±0.14	6.25±0.11	5.25±0.12	5.00±0.11
	Encapsulated	9.15±0.14	8.15±0.20	7.90±0.16	5.83±0.11
5	Free	9.15±0.14	6.90±0.17	6.50±0.16	5.80±0.14
	Encapsulated	9.15±0.14	8.60±0.21	7.90±0.19	6.80±0.17
6.5	Free	9.15±0.14	7.55±0.19	7.20±0.17	7.10±0.18
	Encapsulated	9.15±0.14	8.80±0.17	8.00±0.13	7.94±0.19

*ND: No detected. Results of CFU/g are expressed as mean of triplicates ± standard deviation. Lowest limit of detection was ≤100 CFU/g.

These flocs can result in the formation of a dense and homogeneous network that leads to gelling of the aqueous phase (Ghasemi *et al*., 2017; Wijaya *et al*., 2017). Pectin hydrogels exhibit pH-sensitive swelling characteristics due to deionization of the functional groups in the hydrogel which affects the penetrant transport mechanism of the polymer networks (Güner *et al*., 2018). At the same time, highly swollen hydrogels contain large amounts of unbound water which allows greater solute release, which favors the Lp-LBC to be exposed to the environment (Kim, *et al*., 2003).

3.4 Survival of free and encapsulated bacteria under simulated gastrointestinal conditions

The encapsulated Lp-LBC presented greater viability than free bacteria after simulated gastrointestinal conditions (Fig. 1). In the gastric simulation test the free cells were more sensitive to pH 2 and pepsin exposure, since they presented a decrease from 9.15 to 3.90 Log CFU/g of bacterial suspension, approximately a 5.25 Logs loss. Whereas the encapsulated Lp-LBC presented a lower decrease in viability from 9.15 to 6.10 Log CFU/g of dry capsules, corresponding to a decrease of 3.05 Logs. This effect was similar to that observed during the exposure of the capsules to pH 2, however, it is the addition of digestive enzymes that leads to a great loss of the viability of the encapsulated bacteria. Subsequently, in the intestinal simulations, free cells of Lp-LBC showed a lower final viability of 1.20 Log CFU/g in comparison to the encapsulated bacteria, which presented a viability 4.5 times higher than that

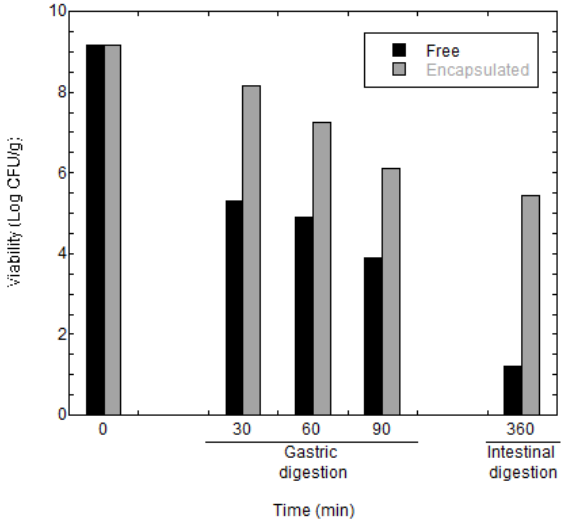

Fig. 1. Viability of microencapsulated *L. paracasei* LBC LYO 10D under simulated gastrointestinal conditions. Results are expressed as mean of Log10 colony forming units per gram (CFU/g). Lowest limit of detection was ≤100 CFU/g.

observed in non-encapsulated cells. The protection and release of viable cell probiotics in the intestine is another of the main objectives of microencapsulation. In this work, the viability of microencapsulated Lp-LBC before simulated gastrointestinal conditions was 9.15 Log CFU/g, a viable cells concentration higher than recommended by FAO/WHO, (2002), to produce a beneficial effect on host health. It has been reported that the protection provided to probiotics by biopolymers is due to strong bonding between wall materials leading to the formation of a waterproof membrane on the surface of the granules, which

reduces the probability of the migration of coating materials. The viability of probiotics was mostly affected by exposure to simulated gastrointestinal conditions; however, the viability of encapsulated bacteria (5.54 Log CFU/g) was greater than free bacteria (1.20 Log CFU/g) at the end of intestinal digestion. The recommended viability of beneficial microorganism cells to be considered probiotic was just reached after gastric juice digestion, but low viability was observed after staying 360 min in the intestinal solution. Secondly, pectin is an aqueous soluble polymer prone to swelling as well as erosion in aqueous medium, leading to premature drug release in the upper gastrointestinal tract and thereby defeating its ability as a colon-specific drug delivery vehicle. In natural conditions, this biopolymer is rapidly degraded by the native microbiota of the colon, being the main characteristic for which its use has been enhanced in the encapsulation of living microorganisms or sensitive bioactive compounds. Hence, pectin is suitable for use as a colon-specific delivery vehicle with minimal degradation in the upper gastrointestinal tract (Wui *et al*., 2010). Based on these results, it can be understood that in addition to the pH value to which both the free and encapsulated cells are exposed, the human digestive conditions affect the viability of free and encapsulated probiotics depending on the residence time and the presence of specific enzymes of each stage of digestion.

3.5 Morphological analysis of the microcapsules spray-dried

Micrographs of the capsules spray-dried at three inlet temperatures showed no obvious visual differences. Example of this is presented in Figure 2, which shows that the dry capsules presented a spherical shape with a serrated surface and a deflated balloon-like shape (ballooning), with a wide distribution of sizes. This distribution of sizes was confirmed by the image analysis, which allowed identify capsules diameters from 1 to 150 μm. These results are similar to those reported by Arepally *et al*. (2020b), who obtained a probiotic powder with smaller size than the droplets formed by encapsulating agents (arabic gum, maltodextrin and combination of both) in emulsion. The decrease in size of the droplets generated in the emulsion is mainly due to the evaporation of water during the drying process. The spray drying, used to obtain probiotic capsules, atomizes the emulsion at high velocity into small droplets of 1-150 μm size, producing a dry powder by injection of hot air

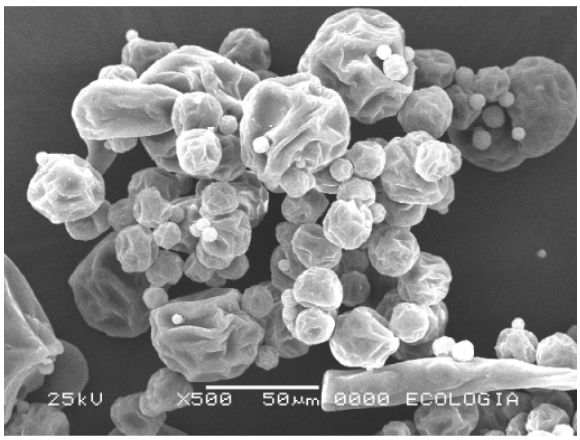

Fig. 2. Scanning electronic micrographs of spray-dried microcapsules of *L. paracasei* LBC LYO 10D, with x500 magnification. The with bar equates to a length of 50 μm.

at temperatures from 150 to 190 ºC. These drying conditions are recognized for producing powdered materials with positive characteristics such as longer shelf life, secure handling (according to their flow properties) and convenience of consumption (Arepally *et al*., 2020a, 2020b). Regarding ballooning, some investigators have suggested that this phenomenon is typical of products obtained by spray-drying, and its presence is due to shrinkage of the particles during drying process, either by the rapid evaporation and high pressure found inside the particles or by diffusion of solutes in the droplet (Nunes *et al*., 2018; Cancino-Castillo *et al*., 2020; Lu *et al*., 2020). Additionally, the absence of visual cracks or fractures on the dry capsule surface suggests appropriated protection of probiotic bacteria due to a minimal or non-existing air permeability of dry capsules (Nunes *et al*., 2018). These features are derived from the correct selection of encapsulating agents, in this case gum arabic/high methoxyl pectin, and a suitable drying process such as spray-drying.

The size of the microcapsules is a parameter that reflects their quality and helps us to predict the level of protection during the gastrointestinal tract and their sensory properties when they are added to a foodstuff. The food industry requires capsules of small size, to avoid creating undesirable sandy and gritty textural properties in the food product in which they are added (Vivek, 2013). Regarding this, it had been reported that a diameter <30 μm is desirable, because larger capsules might cause coarseness of texture in soft food such as iced milk and also do not provide a correct release of the core at the target

site (Choudhury *et al*., 2021). Based on the above information, the capsules obtained in the present work have an acceptable size to be added into a food matrix and the characteristic shape of a product obtained by spray drying. These characteristics suggest a correct protection of the microorganism during storage and its controlled release at specific sites within the intestinal lumen, after gastrointestinal conditions.

3.6 Addition of microcapsules to a fresh panela cheese matrix and sensory evaluation

Fig. 3a shows the viability both free and encapsulated Lp-LBC cells added to the panela cheese, and their survivability during the storage time. The addition of free and encapsulated Lp-LBC cells into fresh cheese aimed to demonstrate the usefulness of the spray-drying in the design and production of new probiotic functional foods. A range of viability of 5.6 and 5.10 Log CFU/g was found for the encapsulated bacteria and free cells added to cheese, respectively. The cheese enriched with the bacterial capsules presented slightly higher moisture content during storage (Fig. 3b), which is reflected in an increase in color parameter b^* and the ΔE during storage time (Figs. 3c and 3d). These results indicate that the capsules addition confer changes in the physical properties of cheese during storage, being more evident after the 10th day of storage. Consumers were questioned about the agreement or disagreement of the sensory attributes such as: general preference, flavour, texture, sandiness, and colour, and they were questioned about the best attribute that defined the samples. The results of the attributes of cheeses added with the encapsulated Lp-LBC, free Lp-LBC cells and without microorganisms (identified as Control), were grouped according to the responses frequency. Figure 4 shows that the Control and cheeses with free microorganisms did not present significant differences ($p>0.05$) in the levels of satisfaction of in each one of the attributes described. The acceptance and preference of the flavour of cheeses with encapsulated Lp-LBC was like that of the cheese's samples with the free Lp-LBC cells and without them. However, the texture and color of the cheeses with the capsules presented the lowest values in the level of satisfaction. Thus, the incorporation of capsules in cheese seems to induce adverse changes in the texture, may be due to the particle size of the capsules.

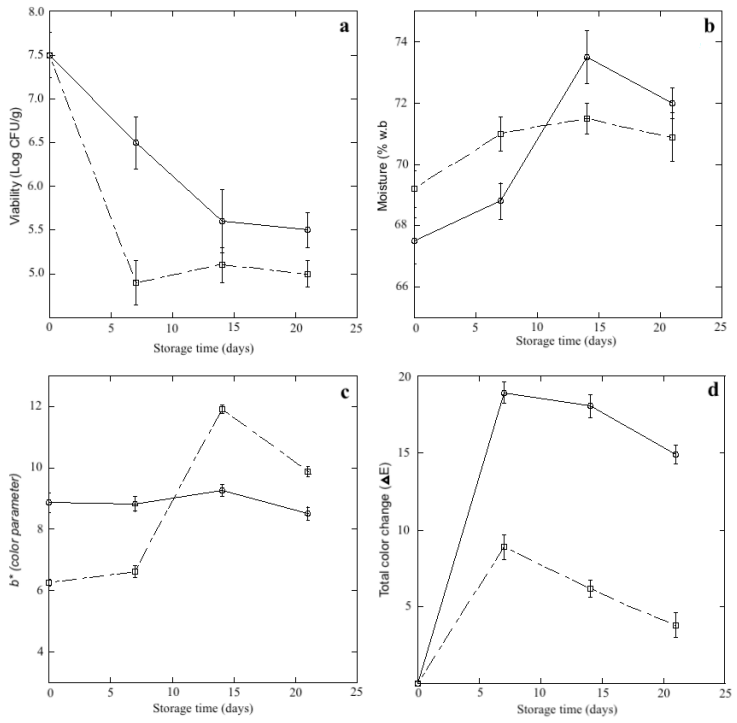

Fig. 3. Viability (a) and physicochemical properties of moisture (b), b^* color parameter (c) and total color change (d) of microencapsulated (o) and free (□) *L. paracasei* LBC LYO 10D added to fresh panela cheese during storage.

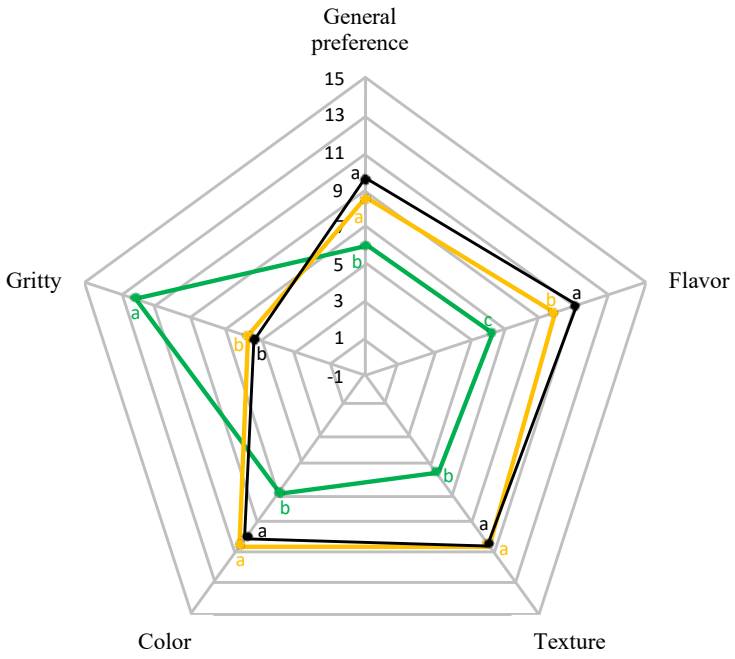

Fig. 4. Comparison of sensory preferences of between fresh panela cheese added with microcapsules (–) and free cells (–) of *L. paracasei* LBC LYO 10D, and without microorganism addition (-). Different letters indicate significant difference ($p<0.05$) between cheese samples in the same sensory preference.

Cheese is the most popular product used as probiotic carrier compared to more acid fermented dairy products such as yoghurt. Most of the different cheeses maintain the viability of microencapsulated microorganisms, as well as to achieve adequate nutritional, technological and sensory properties of the final product (Picciotti *et al*., 2021). However one of the main challenges is to enhance the protection of the probiotic during storage and gastrointestinal digestion. To protect the probiotics viability and to obtain a controlled release of the microorganisms, currently research has focused on the use of microencapsulation techniques, not only to protect, but to add said microorganisms in food matrices that serve as a vehicle for their consumption. In this work, capsules offered greater protection to Lp-LBC during storage in panela cheese as food matrix. Cheeses showed significant changes in moisture content and color during storage. In relation to moisture, the capsules present an absorption up to 15 days, after this time, a decrease in moisture content of both samples of probiotic bacteria. This behavior can be linked to the formation of hydrogels by the pectin present as wall material in the samples of encapsulated Lp-LBC, which after absorption of water from the medium, show a syneresis effect derived from the presence of large pores on capsule surface (Souza Almeida *et al*., 2021). Similarly, the color change of the capsules induces a limited consumption of the product despite its probiotic properties. Another factor that can limit the probiotic cheese consumption is the particle size since it can modify the appearance and mouthfeel attributes of panela cheese (Kavas *et al*., 2021). These effects were confirmed by high values of sandiness conferring adverse characteristics; since the presence of particles with a diameter greater than 30 μm produces unfavourable responses from consumers due to the resulting grainy texture (Adhikari *et al*., 2003; Sandoval-Castilla *et al*., 2010). We found that flavour was not altered by the addition of encapsulated probiotics, but high particle size adversely affected the sensory properties of the product, associated with the sandiness which affected the textural and color properties. Other bacterial encapsulation techniques have also used for probiotic addition to fresh cheeses. For example, Afzaal *et al*. (2019), encapsulated the probiotic strain *Bifidobacterium bifidum* in two hydrogel materials (κ-carrageenan and sodium alginate), by using an internal gelation method. The authors reported undesirable modifications of taste and texture of cheddar cheese added with the probiotic capsules, pointing to the Control cheese

as the sample with the highest acceptance among the consumers. Similarly, Ningtyas *et al*. (2019), used alginate gelation to obtain microcapsules of *Lactobacillus rhamnosus* which were subsequently added to cream cheese. The microgel particles added to cheese led to a firmer and thicker cream cheese, improving its acceptation by the consumers, compared to the cream cheese added with non-encapsulated bacterial strains. Both studies point to gelation as the best technique for obtaining encapsulated probiotics for their subsequent addition in solid dairy products such as cheeses, in which its presence does not represent sensory or structural alterations. Thus, with the aim of counteract the negative effects reported in this studied related to the use of spray dried capsules, it is recommended that future cheese formulations must contemplate parameters such as particle size and texture of probiotic capsules, as well as probiotic strains in order not to damage the acceptability and palatability of the final product by the consumers (Picciotti *et al*., 2021).

Conclusions

A mixture of gum arabic/high methoxyl pectin was used as wall material for the encapsulation of the probiotic *Lactobacillus paracasei* LBC LYO 10D by spray drying, with an encapsulation efficiency of 77% at an inlet temperature of 170 ºC. These conditions generated capsules with flow properties adequate for their transport and storage, with a viability of probiotic bacteria higher than recommended to guarantee its probiotic effect. However, under simulated gastrointestinal conditions, the viability of the capsules decreased significantly as well as their probiotic potential. Despite this, encapsulation generated greater protection compared to free cells. Viability loss during stress conditions was linked to the structure of dry capsules, since effects such as ballooning and heterogeneous sizes, commonly generated by spray drying conditions, induce structural changes that compromise the stability of the capsule. Also, the encapsulating agents such as pectin demonstrated water absorption capacity and syneresis when dry capsules were added to a fresh panela cheese used as food matrix. This water absorption capacity greatly influenced the changes in texture, color and acceptance of panela cheese added with the dry capsules. Therefore, based on the results obtained, it was concluded that the spray drying does not facilitates the incorporation of dry material into fresh and moist dairy products, such as cheeses; so that future investigation related to design of functional dairy foods from encapsulated probiotic should be aimed to development capsules made of polymers with a similar texture and sensory attributes that the food matrix where will be added.

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