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Microencapsulation by spray-drying of *Manilkara zapota* pulp and probiotics (*Lactobacillus fermentum* A15): Assessment of shelf-life in a food matrix

Microencapsulación mediante secado por aspersión de pulpa de *Manilkara zapota* y probióticos (*Lactobacillus fermentum* A15): Evaluación de vida de anaquel en una matriz alimentaria

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Abstract

In the interest of getting a functional and non-dairy food product which improves human health, this research was conducted to obtain an edible gel containing probiotic *Lactobacillus fermentum* A15, and bioactive compounds from an autochthonous Mexican fruit known as chicozapote (*Manilkara zapota*). Probiotic and fruit pulp were encapsulated separately by means of the spray-drying process and later incorporated into the food matrix. Water activity, hygroscopicity, total phenolic compounds, total flavonoids and antioxidant capacity were determined on the pulp fruit microcapsules. Meanwhile viability was measured on the microencapsulated probiotic. Both microparticles were incorporated in a food gel and its shelf-life stability was evaluated by measuring the evolution of the afore-mentioned parameters with time. The viability of probiotics (1×10^7 CFU/mL) was highlighted. The results suggest that this new non-dairy product could be of particular interest for lactose-intolerant consumers and attractive to the new food market demands.

Keywords: Functional food, microencapsulation, chicozapote, probiotics survival, protective agent, non-dairy food.

Resumen

Con el fin de obtener un producto alimenticio funcional y no lácteo que beneficie la salud humana, se realizó esta investigación para obtener un gel comestible que contenga el probiótico *Lactobacillus fermentum* A15 y compuestos bioactivos de una fruta autóctona mexicana conocida como chicozapote (*Manilkara zapota*). Los probióticos y la pulpa de fruta se encapsularon de forma separada mediante la técnica de secado por aspersión y se incorporaron a la matriz alimentaria. En las microcápsulas de pulpa de fruta se determinó la actividad de agua, higroscopicidad, compuestos fenólicos totales, compuestos flavonoides totales y capacidad antioxidante. Por otra parte, en los probióticos microencapsulados se evaluó la viabilidad. Ambas micropartículas se incorporaron en un gel comestible, al cual se le determinó su estabilidad durante el almacenamiento, midiendo los parámetros antes mencionados. En estas medidas se dio un énfasis particular a la viabilidad de los probióticos (1×10^7 UFC/mL). Los resultados obtenidos sugieren que este nuevo producto podría ser un buen sustituto de los productos lácteos para las personas intolerantes a la lactosa vinculadas y puede ser atractivo a las nuevas demandas del mercado.

Palabras clave: Alimentos funcionales, microencapsulación, chicozapote, supervivencia de probióticos, agentes protectores, alimentos no-lácteos.

1 Introduction

Within the group of fruits, those belonging to the *Sapotaceae* family are of great interest due to the nutritional properties and their high antioxidant capacity. *Manilkara zapota*, traditionally known as chicozapote, is an autochthonous fruit from Mexico that has not been studied extensively. It is consumed fresh or processed, and its availability is restricted by season, which limits its consumption (Shui *et al.*, 2004).

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In the other hand, bacteria from *Lactobacillus* genus play an important role in the native microbiota of humans. These probiotics can resist gastric acid, bile salts and pancreatic enzymes. Additionally, they exhibit pathogen inhibition due to their mucosa adhesion in the colon, colonizing the gastrointestinal tract. Regular consumption of *Lactobacillus* promotes a positive balance in gut microbiota (Jayashree *et al*., 2018). Is a fundamental challenge to assure the viability of probiotics like *Lactobacillus* and *Bifidobacterium* specially in non-diary food matrices. The latter are formed by probiotics and prebiotics which can be obtained from fruits pulps, producing in a synergistic effect (Min *et al*., 2018. Non-dairy products are gaining popularity because of their healthy ingredients like living microorganisms (probiotics) and antioxidants (phenolic compounds and flavonoids), which are produced during secondary metabolism of plants, fruits and non-digestible carbohydrates (prebiotics and dietary fiber) (Guimarães *et al*., 2019; Min *et al*., 2018; Nagpal *et al*., 2012; Do Espírito-Santo *et al*., 2011).

Microencapsulation is a technology that has been used to maintain the viability of probiotics and release antimicrobial metabolites, as well as to provide protection to phenolic compounds during food processing and storage (Li *et al*., 2018; Miller *et al*., 2018; Akbas *et al*., 2017; Pérez-Chabela *et al*., 2013). Spray-drying has been used in formulations with fruit pulps because of its simplicity and scalability (Macías-Cortés *et al*., 2020; Robert and Fredes, 2015; Heidebach *et al*., 2012). The latter are consequence of the short exposure time to heat and fast particle drying during the process, which makes it an attractive and well-established unit operation for many applications that can be taken to industrial level. Spray-drying fits well the drying of a variety of compounds including heat-sensitive products. Also, in terms of cost, spray-drying allows a continuous bulk production of dried foods (Villegas-Santiago *et al*., 2020).

The aim of this work was to produce separately microcapsules of *M. zapota* pulp and *Lactobacillus fermentum* A15, by means of spray-drying process. Further characterization of a gel-like food prepared with both microcapsules, included the physicochemical stability, viability of bioactive compounds and probiotics during storage.

2 Materials and methods

2.1 Raw material and chemicals

Fresh *M. zapota* fruit in consumption ripeness degree was purchased from the local market (Guadalajara, Mexico), washed and disinfected with a NaClO solution (2 mg/L) for 5 minutes; then, excess water was drained. Moisture percentage, pH, soluble solids (°Brix), and acidity of the obtained fresh pulp were measured. Later, the pulp was blanched for 3 minutes with distilled water at 95 ºC and cooled into an ice-water bath at 4 ºC for one minute. The pulp was extracted and moisture percentage, pH, soluble solids (°Brix) and acidity were again measured. Phenolic compounds and antioxidant activity were determined in fresh and treated samples; the description of these procedures is presented in following sections. Finally, the pulp was weighed and packed in plastic bags, hermetically sealed, and stored at -20 ºC until use. Maltodextrin (MD) Inamalt 110 (10 DE) was purchased from Compañía Indrustrializadora de Maíz (Guadalajara, Mexico).

Lactobacillus fermentum A15 was provided by Universidad Autónoma de Baja California Sur (Laboratorio de Ciencia y Tecnología de Alimentos). From the stored strain, volumes of 3 to 5 mL of MRS broth at 1% (v/v) were inoculated and incubated for 24 to 48 h at 36 ºC ± 1 ºC. An aliquot was stored with 40% (v/v) glycerol at -80 ºC until use, being a common routine for sample conservation.

All the chemical reagents were analytical grade purchased from Sigma-Aldrich (St. Louis, Mo. USA).

2.2 Microencapsulation of Manilkara zapota fruit pulp by spray-drying

Briefly, fruit pulp was diluted in distilled water (1:2 w/v) and filtrated thrice using a muslin cloth. Experimental conditions were set in a spray dryer (LabPlant, SD-BASIC, UK) with a 0.55 L/h of drying air flow rate. Optimized conditions to obtain microcapsules were applied in study, which are: 21% MD and 130 ºC of inlet temperature and 60 ºC of outlet temperature.

2.2.1.1 Physicochemical properties

Yield percentage was calculated by weighing the total mass of powder obtained per unit of liquid solution fed to the spray dryer.

The dissolution time (DT) of microencapsulates was determined by mixing 5 g of the powder in 25 mL of distilled water at 25 ºC, under strong stirring. DT corresponds to the time needed to observe a full transparent solution. This sample was used for pH (Sper Scientific 850051, China) and °Brix evaluation (Abbe Refractometer DR-A1, Japan). Acidity was expressed in g of citric acid per 100 g of pulp wet basis (g CA/100 g w.b.) (AOAC, 2000).

The moisture determination was calculated according to Tonon *et al*. (2010). Water activity value (a_w) was determined using an Aqualab Pawkit (Decagon Devices, USA) using two grams of sample and placed in a chamber by the dew point principle. Bulk density (g/cm^3) was assessed according to Tonon *et al*. (2010). Water Solubility Index (WSI) was evaluated using the methodology described by Kha *et al*. (2010) with some modifications. Briefly, 1 g of microcapsules and 10 mL of distilled water were mixed for 5 min in a 15 mL centrifuge tube. Then, the tube was centrifuged for 25 min at 2,500 rpm in a Hermle, Labor technik centrifuge Z326. The supernatant was carefully collected in a pre-weighed porcelain capsule and oven dried at a temperature of 95 ºC for 24 h. The WSI was calculed as is described:

$$WSI = \frac{\text{Oven dried sample (g)}}{\text{Initial weight of microcapsules (g)}}]100 \quad (1)$$

The Water Absorption Index (WAI) was calculated following the procedure established by Ahmed *et al*. (2010); briefly, the pellet obtained from the WSI determination, was weighted and the determination was performed as follow:

$$WAI = \frac{\text{Pellet weight (g)}}{\text{Initial weight of microcapsules (g)}} * 100 \quad (2)$$

Finally, hygroscopicity was determined following the methodology proposed by Arrazola *et al*. (2014). Briefly, several Petri dishes containing 1 g of microcapsules were stored at 25 ºC, inside a desiccator having a relative humidity of 75.4%. A saturated NaCl solution was placed into the desiccator to keep the humidity constant. Samples were weighed every week until constant weight was observed. The results are expressed as g of water per g of dried basis (g/g).

Color variation of samples was evaluated using a colorimeter CIELAB scale (ColorFlex, USA), using total color differences (ΔE^*_{ab}), calculated from:

$$\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

where ΔL^*, Δa^* and Δb^* is the difference in color between each standard (maltodextrin) and that measured on powder samples.

2.2.1.2 Quantification of bioactive compounds

The extraction of bioactive compounds was carried out using 2 g of microcapsules of *M. zapota* pulp with 20 mL of a methanolic solution at 60% (v/v), at constant stirring (120 rpm) for a period of 24 h. Subsequently, the solution was centrifuged at 3000 rpm for 10 min. The supernatant was recovered and the pellet was washed twice with 10 mL of a methanolic solution at 60% (v/v). The supernatants were pooled and filled up to 50 mL with distilled water, covered with aluminum foil and stored in darkness. This extract was used to calculate total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA).

A modified Folin-Ciocalteau method was used to calculate the total phenolic compounds (TPC) (Singleton *et al*., 1999). Aliquots of 200 μL from the above extract were mixed with 1.5 mL of Folin-Ciocalteau reagent diluted in distilled water (1:10), the reaction was stopped after 5 minutes and immediately neutralized with 1.5 mL of $NaHCO_3$ 6% (w/v). Samples were protected against light for 60 min. Absorbance was recorded at 725 nm by using an UV-VIS spectrophotometer (UNICO, USA). The standard curve was obtained using a gallic acid solution with concentration in the range of 0-250 μg/mL. The results are expressed as mg of gallic acid equivalent per 100 g of sample on dried basis (mg GAE/100 g d.b.).

Total flavonoid content (TFC) was determined according to Dewanto *et al*., (2002). Aliquots of 0.25 mL from the extract were mixed with 1.25 mL of distilled water, followed by the addition of 75 μL of NaNO2 5% (w/v). The resulting mixture reacts during 6 min, and 150 μL of 10% AlCl3 10% (w/v) were added and further incubation for 5 min takes place. After incubation, 0.5 mL of 1M NaOH solution were added. The mixture was filled up to 2.5 mL with distilled water. The absorbance was measured at 510 nm and calibration curve was determined using catechin with concentration 0-1000 μg/mL. Data were expressed as mg of catechin equivalent per 100 g of sample on dried basis (mg CAE/100 g d.b.).

Antioxidant activity (AA) was assessed using the free radical DPPH method (Rajauria *et al*., 2010; Jimenez-Escrig *et al*., 2001). The results were evaluated in terms of efficient concentration (EC50), which is the concentration of the extract until 50% of the DPPH radical reduction is achieved. Data were

expressed as μg of Trolox per 100 g of dried basis (μg of Trolox /100 g d.b.).

2.2.2 Microencapsulation of probiotic strain

Lactobacillus fermentum A15 was incubated overnight at 1% (v/v) in 100 mL of MRS broth at 37 ºC. Cell concentration was adjusted to $10^8 - 10^9$ CFU/mL by means of the optical density, measured at 600 nm using a spectrophotometer (Unico, USA). Cells were retrieved by centrifugation at 3000 rpm for 10 min at 25 ºC. The supernatant was discarded and the pellet was washed twice with PBS. Cell pellet was re-suspended in whey protein according to the quantity reported in Table 1a.

To obtain the suspension, all the components reported in Table 1a were mixed. Then, sterile distilled water was added until viscosity reached 100 cP (centipoise). The viscosity was measured using a viscometer (Brookfield, USA) with a needle number 6 at 25 ºC. Immediately, samples were spray-dried using an inlet temperature of 130 ºC, whereas the outlet temperature was 60 ºC. The powder was collected, packed in sealed plastic bags and refrigerated at 4 ºC until use.

2.2.3 Microencapsulated probiotics incorporation into food matrix

Table 1b shows gel composition; all the compounds were added in a beaker with continuous stirring at 50 ºC. The obtained gels were stored in refrigeration at 4 ºC for a period of 6 weeks. This period of time is associated with the viability of the microorganisms in the sample as will be described in the results section. Moisture, water activity (a_w), pH, soluble solids (°Brix), acidity, viscosity, TPC, TFC, and viability of probiotics were determined in these samples.

2.2.3.1 Viscosity

Viscosity was measured according to Artiga-Artigas *et al*., (2017); 250 g of gel were placed in a beaker and viscosity was determined in cP using a viscometer (Brookfield, USA) with a needle number 6, at 25 ºC.

Table 1. Formulations of: a) Suspension of *Lactobacillus fermentum* A15 before spray-drying and, b) Edible gel of *Manilkara zapota* with *Lactobacillus fermentum* A15.

Suspension of *Lactobacillus fermentum* A15 (a)		*M. zapota* and *Lactobacillus fermentum* A15 gel (b)	
Components	**Composition**	**Components**	**Composition**
Inulin	5 wt%	Water	69.27 wt %
Sodium alginate	1 wt %	Sucrose	10 wt %
Maltodextrin	5 wt %	Glucose	3 wt %
Whey protein	1.5 wt %	Inulin	2 wt %
***Lactobacillus fermentum* A15**	$1x10^8$ UFC/mL	Sodium alginate	2 wt %
		Citric acid	1 wt %
		Microencapsulated *M. zapota*	12.7 wt %
		Yellow dye	0.024 wt %
		Carmine dye	0.003 wt %
		Microencapsulated *Lactobacillus fermentum* A15	$1x10^7$ UFC/mL

2.3 Shelf-life study

2.3.1 Microencapsulated Manilkara zapota pulp

Samples stored at 25 ºC were evaluated for 6 weeks. At each sampling point, the hygroscopicity, TPC, TFC and AA, were evaluated according to Flores *et al*., (2014).

2.3.2 Microbial viability

The powder was reconstituted with PBS (1:10 w/v) and the suspension was maintained at 25 ºC for 12 min in a vortex mixer to disintegrate capsules and release the cells. Probiotics viability (*Lactobacillus fermentum* A15) was counted at 0, 15, 30 and 45 days of storage at 4 ºC, using 3M^{TM} Petrifilm^{TM} plates for BAL counting. Colony counts were expressed as CFU/g.

2.4 Scanning electron microscopy (SEM) and differential scanning calorimetry (DSC)

The morphology of microcapsules was observed by field-emission scanning electron microscopy (FESEM), using a MIRA 3 microscope (Tescan Instruments, Czech Republic). The glass transition temperature (Tg) was determined by differential scanning calorimetry (DSC), using a Discovery calorimeter (TA Instruments, USA). The DSC results were analyzed with the TRIOS® software v4.40. Thermograms were registered from 10 to 100 ºC, with a heating ramp of 10 ºC/min under nitrogen atmosphere. The first inflection point of the glass transition was considered as the characteristic transition temperature.

The glass transition temperature and water activity were determined to assure processing and manufacturing conditions. Moreover, these parameters are related to the product quality, as well as the stability and safety of any dehydrated food. Specifically, glass transition temperature was determined because a change from the glassy to the rubbery state promotes significant variations on the mechanical properties of powdered foods. In this case, caking, stickiness, structural collapse and crystallization of sugars can occurred. Furthermore, transition to the rubbery state, decreases the storage time in weeks, days or even hours (Carter and Smith, 2012).

2.5 Data analysis

All the experiments were conducted in duplicate and standard deviation (SD) was calculated. Error bars in the graphs denote ± SD and were drawn with Origin Pro 8.0 software.

3 Results and discussion

3.1 Physicochemical and antioxidant properties of fresh, blanched and microencapsulated M. zapota pulp

Table 2 shows the physicochemical properties for *M. zapota* pulp (fresh and blanched) and microencapsulated *M. zapota* fruit pulp. It can be observed that the blanching process does not affect the properties of fruit pulp regarding moisture content, pH and acidity levels. Thermal blanching improves the extraction of bioactive compounds because the structural changes are promoted by cell membrane disruption and contribute to the inactivation of polyphenol oxidase (PPO). However, a reduction in the values of TPC, TFC and AA is observed, due to the degradation produced by using a high blanching temperature. However, in order to decrease the loss of bioactive molecules, a less harmful process than blanching can be used, for instance scalding in hot water for removing the waxy layer.

In this sense, it is worth to mention that blanching was effective in removing the waxy layer or latex sap present in the fruit surface, which interferes in the moisture transfer phenomena occurring during drying process (Reyes-Gómez *et al*., 2018; Xiao *et al*., 2017).

Moreover, from Table 2, a yield of 57.77% obtained by using the spray-drying conditions of this study, is one of the highest values achieved for plant and fruits extractions (da Silva *et al*., 2019; Looi *et al*., 2019). About a moisture content of 6.65%, measured on microencapsulated *M. zapota* pulp, is attributable to hydrophilic groups from MD (Niamnuy *et al*., 2019). Moreover, high percentages of MD determine the powder moisture content, because water molecules cannot completely diffuse through MD molecules, which is a non-hygroscopic compound. Consequently, the addition of MD provides low hygroscopicity to the desired final product (Goula and Adamopoulos, 2010; Fontes *et al*., 2014).

Table 2. Physicochemical properties of fresh or blanched *Manilkara zapota* (above) and, microencapsulated *M. zapota* pulp and the probiotic *L. fermentum* A15 (below).

Parameter	*Manilkara zapota*	
	Fresh	**Blanched**
Moisture (%)	79.89 ± 2.71	78.46 ± 1.52
pH	1.6 ± 0.00	1.7 ± 0.00
Acidity (g citric acid / 100 g pulp w.b.[1])	0.46 ± 0.00	0.44 ± 0.00
Total Polyphenolic Content (mg GAE[2] / 100 g d.b.[3])	9726.88 ± 743.62	5284.95 ± 297.04
Total Flavonoid Content (mg CAE[4] / 100 g d.b.)	566.78 ± 18.67	442.94 ± 20.22
Antioxidant Activity (DPPH, µg Trolox / 100 g. d.b)	80.92 ± 6.19	69.54 ± 3.26

	Microencapsulated *Manilkara zapota* pulp	**Microencapsulated *Lactobacillus fermentum* A15**
Yield (%)	57.77 ± 10.36	9.93 ± 0.50
Moisture (%)	6.65 ± 1.70	7.56 ± 0.26
Water activity (a_w)	0.31 ± 0.11	0.46 ± 0.03
Bulk density (g/cm^3)	0.67 ± 0.03	0.47 ± 0.01
Water Solubility Index (%)	90.71 ± 4.48	51.59 ± 12.14
Water Absorption Index (%)	66.03 ± 6.08	1.94 ± 0.36
Dissolution time (s)	138 ± 25.46	710 ± 10
Color, ΔE	3.03 ± 0.33	Not determined
Total Polyphenolic Content (mg GAE[2] / 100 g d.b.[3])	377.30 ± 7.69	Not determined
Total Flavonoid Content (mg CAE[4] / 100 g d.b.)	184.72 ± 2.44	Not determined
Antioxidant Activity (DPPH, µg Trolox / 100 g. d.b.)	8.84 ± 0.11	Not determined

(1) w.b.: wet basis; (2) GAE: Gallic Acid Equivalent; (3) d.b.: dried basis; (4) CAE: Catechin Equivalent.

The water activity value (a_w) of microcapsules measured in this work was 0.31. This value is commonly obtained when an inlet air temperature of around 130 ºC and MD as carrier agent are used in spray-drying process. a_w values lower than 0.6 mitigates the deterioration of microcapsules produced by microorganisms and biochemical reactions (Mishra *et al*., 2017; Kha *et al*., 2010). Regarding bulk density, an average value of 0.67 g/cm^3 measured in this work can be attributed to the increase on the total solids feed, leading to a higher sample viscosity. Furthermore, higher concentrations of MD produce spherical particles of thicker walls or solid spheres, which have higher bulk densities (Jumah *et al*., 2000).

The ability of the powder to be dissolved in water is represented as WSI, which in our samples was 90.71%. Meanwhile, the ability of a sample to re-associate with water under limited water conditions

is represented as WAI (Water Absorption Index) (66.03% in this work). The high value of WSI is related to its low protein content, which is attributed to the low protein content of fruit (Nishad *et al*., 2017).

The WAI of *M. zapota* microcapsules was 66%, similar to that reported by Ahmed *et al*. (2010). This parameter is associated to the ability of MD to form external layers over the surface of droplets, promoting their stickiness by the transition to the glassy state. On the other hand, the dissolution time recorded in this work (138 s) is inversely related to WSI, which means that high WSI values resulted in shorter dissolution times. About the color evaluation of samples, MD was used as a blank, which serve to determine differences in color. ΔE value calculated in this study was at approximately 3.03, which is within the threshold value of ΔE (>3) from which a consumer identifies a change in color. It is worth to mention that smaller changes in color cannot be noticed by a human eye (Fontes *et al*., 2014).

3.2 Shelf-life of Manilkara zapota microcapsules obtained by spray-drying

The stability of powders was evaluated in terms of hygroscopicity, on samples preserved at 25 ºC for 60 days, at a relative humidity of 75.4%; Fig. 1a shows the corresponding graph. It can be observed that the maximum value was reached during the first week, and further stabilization is related to the protective role of MD. Hygroscopicity can be explained in terms of glass transition temperature (Tg), because an inverse relationship between Tg and hygroscopicity has been reported in the literature. Then, high Tg is associated to powders of low hygroscopicity; in particular, several studies have demonstrated that MD displays this behavior (Ramakrishnan *et al*., 2018). The Tg curve obtained in this work can be observed in Fig. 3.

Regarding the results obtained from bioactive compounds, they correspond to powders preserved at 25 ºC for 35 days. The TPC values were in the ranged of 325.97 to 377.30 mg GAE/100 g of dry basis, whereas the loss percentage of polyphenols was 13.6%, which can be observed in Fig. 1b, left y-axis. The range of flavonoid content was from 175.06 to 184.72 mg CAE/100 g of dry basis, with a loss of 5.23%, as shown in Fig. 1b, right y-axis. Besides, the change on AA was from 8.84 to 7.82 μg Trolox/100 g of dry basis, reaching 50% of inhibition; a loss percentage of 11.53 can be seen in Fig. 1c. In this regard, the results obtained in this work display a similar trend when MD is employed as wall material. In this sense, MD has a great capacity to stabilize bioactive compounds (Bakowska-Barczak and Kolodziejczyk, 2011).

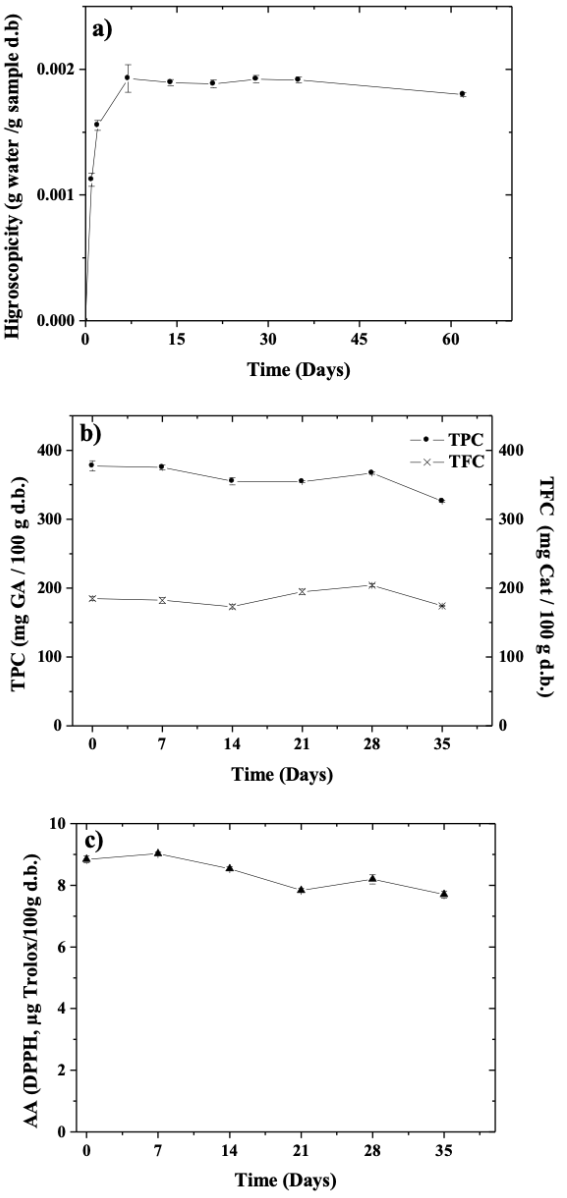

Fig. 1. Shelf-life of microencapsulated *M. zapota* pulp (a) higroscopicity, (b) Total Polyphenolic Content (TPC) and Total Flavoinod Content (TFC) and, (c) Antioxidant Activity (AA). Samples were stored at 25 ºC. Each point represents a mean value of 2 measurements. Errors bars denote standard deviation.

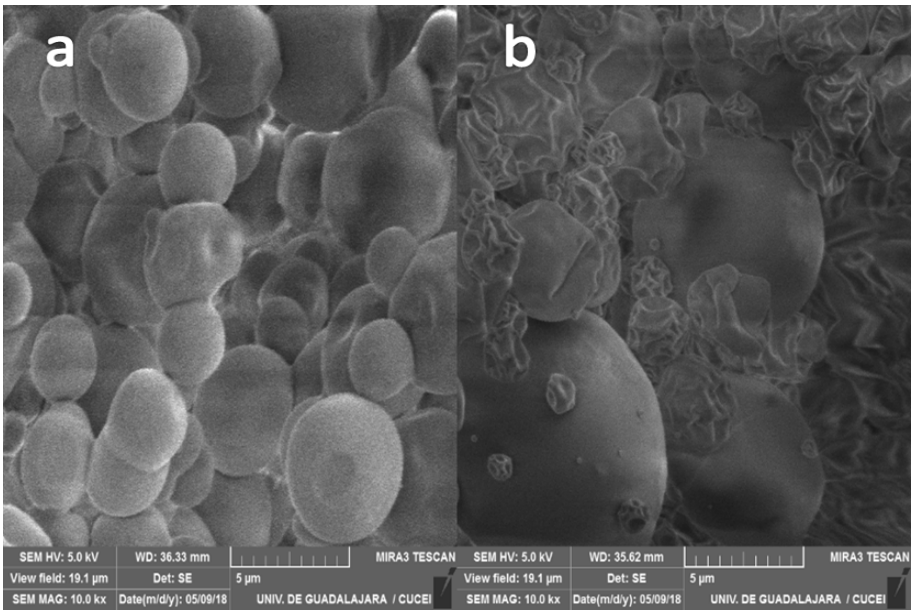

Fig. 2. SEM micrographs of: a) Microencapsulated *M. zapota* and, b) Microencapsulated *Lactobacillus fermentum* A15.

3.3 Morphology and glass transition temperature

Fig. 2a shows the morphology of *M. zapota* microcapsules, which corresponds to microspheres without roughness and absence of collapsed particles. The size distribution ranged from 2 to 5 μm. In general, the morphology observed in Fig. 2a is typical of powders of fruit pulps obtained by the spray-drying process (Begum and Deka, 2016). Fig. 2b shows the morphology of probiotic microcapsules, which present swollen and shrunken particles, but not cracked. This microstructure is consequence of using a low and medium inlet air temperatures in the range of 74-150 ºC. However, when higher inlet air temperatures are used (153-200 ºC), the capsules display porosity and stiffness, which means that capsule rupture can be involved (Pérez-Alonso *et al*., 2015). In both samples, encapsulated fruit pulp and probiotic microspheres, the absence of fractures is remarkable, revealing the formation of a strong physical barrier of low gas permeability, which delays unwanted oxidation or release of probiotics or bioactive compounds. These results indicate that microencapsulation was an effective process to keep the integrity of the target compounds (Corrêa-Filho *et al*., 2019; Fazilah *et al*., 2019).

As it was mentioned before, sticky materials are the result of a change to the amorphous state, from glassy to rubberlike liquid form. In this sense, the glass transition temperature (Tg) is a second-order transition which represents a variation of the specific heat capacity (ΔCp), and for practical purposes, it is a good parameter to determine the quality and stability of the final microencapsulated product (Huang *et al*., 2014).

Fig. 3 shows typical heat flow vs. temperature graphs obtained from our samples, where the curve a) corresponds to microencapsulated *M. zapota* and b) was recorded from microencapsulated probiotic. Both curves display an inflection (Tg, midpoint) which represents the glass transition temperature.

The onset point for curve a) was 48.59 ºC, with a midpoint at 49.06 ºC and endpoint at 49.23 ºC. For curve b) these values were: 48.51 ºC, 48.86 ºC and 49.11 ºC, respectively. The onset Tg of glass transition plays a key role in food processing since above this temperature, products are subjected to adverse changes in their properties (Zaitoon *et al*., 2016). Moreover, for fruit juices which have a low molecular weight, the influence of sugars in pulps promotes a sticky behavior, associated with their glass transition temperature (Hashib *et al*., 2015). On the other hand, bacterial protection by dehydration phenomena is explained by the water replacement theory, the hypothesis of hydration forces or the vitrification theory (Menzink *et al*., 2017; Dianawati *et al*., 2013; Sakurai *et al*., 2008).

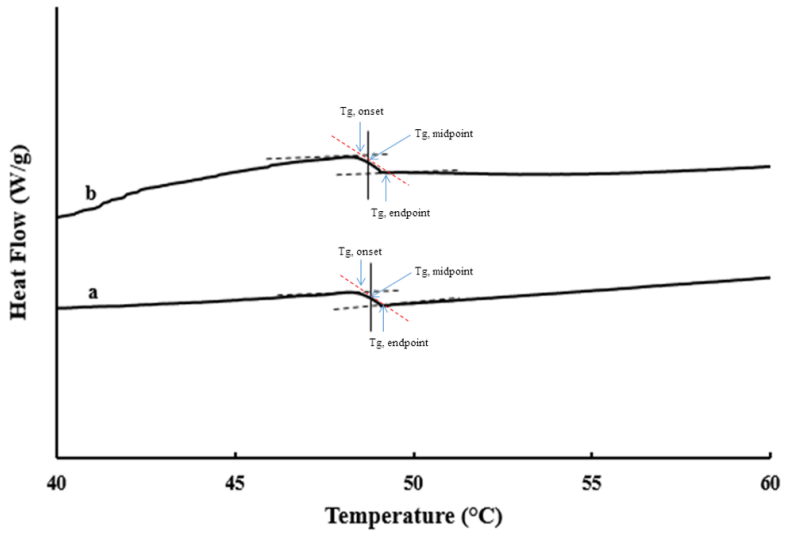

Fig. 3. DSC thermograms of: a) Microencapsulated *M. zapota* and, b) Microencapsulated *Lactobacillus fermentum* A15.

Table 3. Physicochemical and antioxidant properties of edible gels: i) *Manilkara zapota* without encapsulation (*Blank*), ii) microencapsulated *M. zapota* (*MCG*) and, iii) microencapsulated *M. zapota* and probiotic (*MCG + P*) at 0 and 42 days of storage time (average values, n=2).

Samples	Moisture (%)	a_w	Acidity g CA[1]/100 g	pH	Soluble solids (°Brix)	Viscosity (cP)	TPC mg GAE[2]/100 g w.b.[4])	TFC (mg CAE[3] /100 g w.b.)
Time: 0 days								
Blank	79.1±0.26	0.99±0.01	1.11±0.15	2.9±0.00	2.35±0.21	4740±87.2	0.43±0.88	0.05±0.004
MCG	61.85±0.030	0.99±0.00	1.14±0.10	3.1±0.00	5.35±0.21	17716±40.4	147.8±2.08	57±0.00
MCG+P	70.95±0.51	0.99±0.00	0.81±0.14	2.70±0.14	3.75±0.07	5880±749.4	16.32±0.56	18±0.00
Time: 42 days								
Blank	77.60±0.01	0.985±0.001	2.90±0.25	2.90±0.09	2.90±0.20	7213±740	0.1±0.89	0.01±0.001
MCG	66.45±1.55	0.975±0.003	3.05±0.50	3.05±0.02	3.05±0.30	12516±616	131.75±5.59	45±0.03
MCG+P	73.60±1.40	0.965±0.005	3.95±0.05	2.95±0.10	3.95±0.15	7866±173	16.50 ±1.50	35±0.02

(1) CA: Citric Acid; (2) GAE: Gallic Acid Equivalent; (3) CAE: Catechin Equivalent; (4) w.b.: wet basis.

Therefore, the vitreous state (amorphous) of food ingredients and probiotics will become sticker after decrease on their moisture content. In this work, the glass transition temperature of microencapsulates was above room temperature, which is associated to the protective role of maltodextrin. On the other hand, for inulin, sodium alginate and whey protein a Tg onset of around 48.5 ºC has been measured, which indicates that bioactive compounds and probiotic cells are shielded by a glassy matrix, with improved stability of their physicochemical properties.

3.4 Antioxidant and physicochemical properties of edible gels

Table 3 shows the physicochemical and antioxidant properties of: 1) gel made with *M. zapota* without encapsulation (*Blank*), 2) microencapsulated *M. zapota* gel (*MCG*) and 3) microencapsulated *M. zapota* and probiotic gel (*MCG + P*) at the end of storage time (42 days).

Regarding the a_w parameter, which is related to shelf-life, the values followed the next order: (*Blank* > *MCG* > *MCG + P*), due to the structure of core-wall

materials. It is noticeable that the three edible gels have range values between 0.97 and 0.99, which are commonly measured on fresh *M. zapota* pulp (Salleh *et al*., 2017).

The final acidity of *MCG + P* gel (3.95) was higher than that measured on the blank sample (2.90), probably due to probiotic fermentation. However, no significant change of pH was noticed on these samples (2.90 and 3.05, for *MCG + P* and blank samples, respectively). According to the literature, the growth and viability of probiotic bacteria in fruits beverages and food matrixes depend on the bacterial strain. This means that if pH decreases, a negative impact on probiotic viability can be observed (Shori, 2016). In relation to soluble solids, a significant increase in the order *MCG + P* > *MCG* > *Blank* was detected, possibly due to the amount of sugars contained in these samples. In addition to that, an increase on viscosity was observed by decreasing the moisture content.

It can also be mentioned that TPC and TFC were not detected on *Blank* gel; however, they were found in *MCG*, thus it is possible to conclude that the microencapsulation of *M. zapota* pulp was favorable for the protection of the bioactive compounds. On the other hand, *MCG+P* showed a decrease on the amount of TPC and TFC, probably due that they served as a substrate for probiotics (Reddy *et al*., 2018).

3.5 Survival of probiotic bacteria

Cell survival of encapsulated probiotics and edible gel with microencapsulated *M. zapota* and *Lactobacillus fermentum* A15 (*MCG + P*) were > 1.0×10^7 CFU/g (Table 4), at the end of 45 days of storage. Hence, it is highly probable that their consumption as functional probiotic food remained viable in the gut (1.0×10^6 CFU/g) and display their beneficial effects.

Table 4. Average of probiotic Log CFU counts (n=3) at 4°C.

Time (days)	Log CFU/g	
	Microencapsulated *Lactobacillus fermentum* A15	*MCG+P*
0	8.89 ±0.65	7.47 ±0.19
15	8.42 ±0.47	7.34 ±0.12
30	8.22 ±1.03	7.23 ±0.10
45	8.56 ±0.72	7.00 ±0.18

It is well known that the growth of specific colonic bacteria, such as *Lactobacillus* spp., has healthy results in humans, i.e. producing a decrease on blood glucose, regulation of lipid homeostasis and immunomodulation (García-Gamboa *et al*., 2020).

Conclusions

Our results indicate that fruit pulp from *M. zapota* is rich in bioactive compounds, and their microencapsulation by spray-drying process using MD allowed their preservation during storage conditions. Furthermore, the survival of *Lactobacillus fermentum* A15 microcapsules during its shelf-life shows acceptable physicochemical parameters that assure product applicability. Thus, the combination of these microcapsules in an edible gel resulted in an interesting and healthy proposal of a non-dairy and functional food, which may be of particular importance to people with lactose intolerance, dairy allergies or gut microbiota disorders, but for all consumers as well.

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