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Original Article

Minimally processed yam beam roots fortified with probiotics and phenolic compound from microencapsulated green coffee

Jícama mínimamente procesada fortificada con probióticos y compuestos fenólicos de café verde microencapsulado

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ABSTRACT

Yam beam (*Pachyrhizus erosus* L.) root, commonly known as jicama, is widely consumed by health-conscious individuals due to its low caloric content, However, its nutritional value is relatively low. To enhance these nutritional properties, jicama can be supplemented with probiotics and antioxidant compounds. In this study, the jicama pieces were coated with an edible layer containing microencapsulated *Lactobacillus acidophilus*, *Bifidobacterium* spp. and phenolic compounds derived from green coffee, which were microencapsulated using a double spray drying technique. The probiotics and phenolic compounds were dried using double spray drying with chitosan at 120 and 140 °C. The results showed that the inlet air temperature did not have a statistically significant effect ($p \geq 0.05$) on the encapsulation efficiency of probiotics, chlorogenic acid and caffeine content, or antioxidant activity expressed as IC_{50} value (110 - 116 µg/mL). After 6 d of storage at 4 °C, the jicama supplemented with the microcapsules containing *Lactobacillus acidophilus* and *Bifidobacterium* spp. exhibited a reduction in microbial viability by 1 and 2 log CFU/g, respectively. However, the addition of microcapsules allowed a higher concentration of phenolic compounds than the control group. Minimally processed jicama containing microcapsules with probiotics and phenolic compounds could be a functional food, and the reported procedure could be applied for industrial purposes.

Keywords: edible coating; antioxidant compounds; spray-dried.

RESUMEN

La raíz de ñame (*Pachyrhizus erosus* L.) comúnmente llamada jícama, es consumida por personas preocupadas por su salud ya que tiene bajo contenido calórico y, a pesar de que contiene algunas vitaminas, su contenido nutrimental es bajo. Para mejorar estas propiedades nutricionales, la jícama puede ser suplementada con probióticos y compuestos antioxidantes. En este estudio, la jícama fresca en trozos fue recubierta con microcápsulas que contenían *Lactobacillus acidophilus*, *Bifidobacterium* spp. y compuestos fenólicos de café verde, los cuales fueron microencapsulados mediante secado por aspersión doble. Los probióticos y compuestos fenólicos se secaron mediante doble secado por aspersión con quitosano a 120 y 140 °C. Los resultados indicaron que la temperatura de entrada del aire no afectó estadísticamente ($p \geq 0.05$) la eficiencia de encapsulación de los microorganismos, el contenido de ácido clorogénico y cafeína, ni la actividad antioxidante expresada como IC_{50} (110 - 116 µg/mL). Después de 6 d de almacenamiento a 4 °C, en la jícama adicionada con las microcápsulas, la viabilidad de *Lactobacillus acidophilus* y *Bifidobacterium* spp. tuvieron una reducción de 1 y 2 log UFC/g, respectivamente. Sin embargo, la concentración de compuestos fenólicos fue superior que en la jícama del grupo control. La jícama que contiene las microcápsulas con probióticos y compuestos fenólicos podría ser un alimento funcional, y el procedimiento desarrollado podría aplicarse con fines industriales.

Palabras clave: recubrimiento comestible; compuestos antioxidantes; secado por aspersión

INTRODUCTION

In 2022, around 7100 Ha in México were planted with *Pachyrhizus erosus* L. (jicama) with an average production of 24 tons/Ha (SIAP, 2022). Jicama is a legume; the edible structural organ of this plant is the root, which is consumed fresh. The root is low in calories (40 cal) and contains vitamins, minerals and starch (Ramírez-Balboa *et al*., 2023). Although jicama contains most essential amino acids, vitamins and minerals (Duke, 1992), these nutritional values are relatively low. Therefore, jicama is a product that can be supplemented with other bioactive compounds. In this sense, antioxidant compounds and probiotics have been used to fortify other products (Granato *et al*., 2020).

Phenolic compounds exhibit significant biological activities, including antimicrobial, anti-inflammatory (Albuquerque *et al*., 2021), and antitumoral (Heleno *et al*., 2015), among others. Granato *et al*. (2020) reported that an increased intake of natural phenolic compound antioxidants is associated with a reduced risk of coronary disease. While phenolic compounds are present in many fresh foods, they can also be incorporated into foods during processing. In that sense, coffee is widely recognized as a functional food with antioxidant properties, primarily due to its phenolic compounds, as noted by Jeszka-Skowron *et al*. (2016). Although coffee is mostly consumed processed, green coffee has been reported to be a rich source of phenolic compounds, such as chlorogenic acids, hydroxycinnamic acids, caffeine, and caffeic acid

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(Macheiner *et al*., 2019), among others. However, these compounds are easily oxidized during processing and storage, which reduces their effectiveness. Due to the importance of these compounds for human health, researchers are interested in developing systems that protect bioactive compounds during co-administration (Bednarska and Janiszewska-Turak, 2020).

In the context of improving people's health, researchers are looking for new strains of probiotics, as these have been shown to provide health benefits to the host, as demonstrated by Ramírez-Pérez *et al.* (2023) in *in vivo* studies using Wistar rats. Probiotics are live microorganisms that, when administered in adequate amounts, promote a benefit in the health of the host (Ramírez-Pérez *et al*., 2022). Probiotics can help treat gastrointestinal diseases (Fragnant *et al*., 2023), reduce serum cholesterol and heart disease (Oniszczuk *et al*., 2021), regulate glycemic indexes (Rezazadeh *et al*., 2019), control urogenital tract infections (Nader-Macías and Juárez, 2015) and stimulate the immune system (Salami *et al*., 2019). Two common probiotic microorganisms are the bacteria from the genera *Bifidobacterium* and *Lactobacillus* (Ramos-Clamont *et al*., 2013; Yao *et al*., 2019). To provide these benefits, probiotic bacteria must be present with a minimum of 10^6 to 10^7 colony-forming units (CFU) per g or mL of food (FAO/WHO, 2006). However, factors such as stress produced during their management, storage and transit through gastrointestinal tract can decrease their viability (López-Fernández *et al*., 2019; Pupa *et al*., 2021).

To avoid the loss of probiotic viability and to protect phenolic compounds against adverse environmental conditions such as light, moisture, and oxygen, microencapsulation processes are often employed. Several microencapsulation processes have been developed for this purpose, which involve trapping the bioactive compound within a coating material (Reque and Brandelli, 2021). Spray drying is the most widely used technique for protecting probiotics (Russo *et al*., 2022) and phenolic compounds. Because it is difficult for a single encapsulant matrix to have all the required characteristics, it is common to use carbohydrates, proteins and polysaccharides at different ratios (Navarro-Flores *et al*., 2020) or multilayers of these (Abrahão *et al*., 2019; Pupa *et al*., 2021). Chitosan is one of the most promising coating materials used for microencapsulation to improve the stability of phenolic compounds and probiotics (Pupa *et al*., 2021). Microcapsules obtained by spray drying with chitosan are very stable in storage and demonstrate controlled release characteristics because of their low solubility at neutral pH (Flores-Belmont *et al*., 2015).

Although the separate microencapsulation of phenolic compounds and probiotic has been reported in several studies, few have evaluated the effect of double microencapsulation by spray drying on cell viability, phenolic compounds content and the properties of the microcapsules. Additionally, only a limited number of papers have explored the used of these microcapsules in the development of functional foods. Given that jicama has limited attractive nutritional characteristics but is consumed for its low caloric content, this research aimed to determine the effect of inlet air temperature on the properties of microcapsules obtained by single and double spray drying. Moreover, the effect of the addition of probiotics and phenolic compounds from green coffee microencapsulated in coating form on the microbiological, physicochemical, and sensory properties of minimally processed jicama roots was tested.

MATERIAL AND METHODS

Microorganisms and conditions of cultivation

Lactobacillus acidophilus and *Bifidobacterium* spp. (Vivolac, Mexico) were reactivated in Man Rogosa Sharpe (MRS) broth supplemented with 0.05 % (w/v) cysteine, and incubated at 38 °C for 20 h. Subsequently, cells were centrifuged at 4500 rpm for 15 min, at 4 °C. The pellet containing the cells was washed twice with 0.9 % (w/v) saline solution and centrifuged using the same conditions (Odila *et al*., 2016).

Preparation of green coffee extract

The green coffee beans were ground and then passed in a sieve 40 (0.420 mm) to produce green coffee powder. To obtain the green coffee extract, the methodology of Budryn *et al*. (2013) was employed with some modifications. Briefly, the green coffee powder was mixed with distilled water at a 1:5 (w/v) ratio and heated at 90 °C for 1 h. Subsequently, the solution was filtered using filter paper (0.16 mm pore size). Finally, the green coffee extract (GCE) was stored in amber jars at 4 °C until use.

Encapsulation of probiotics and phenolic compounds

The double microencapsulation of microorganisms and coffee extract was performed following the methodology proposed by Flores-Belmont *et al*. (2015), with some modifications. In the first step, an aqueous of gelatin-maltodextrin (1:25) solution, at 26 % (w/w), was prepared in an ascorbic acid solution at 1 % (w/v). The GCE was added to a final concentration of 4.2 mg gallic acid equivalent/mL. *Lactobacillus acidophilus* and *Bifidobacterium* spp. were added to a final concentration of 10^9 and 10^8 CFU/mL, respectively. Subsequently, the mixture was homogenized using an Ultra Turrax T-25 Basic Homogenizer at 4500 rpm for 5 min. The mixture was fed into a spray dryer (BUCHI Mini B-290, Flawil, Switzerland) at a constant flow of 14 mL/min, and two inlet air temperatures, 120 °C and 140 °C, were evaluated, with an outlet air temperature of 50 °C. The microcapsules obtained in the first spray-dried process were hydrated and dried by spray drying in a second step. For this, 10 g of the microcapsules were added to 100 mL of a 0.5 % (w/v) chitosan solution prepared in 1 % (v/v) acetic acid, and the mixture was subjected to the drying process following the same conditions as the first step. Finally, the microcapsules were stored in vacuum-sealed metal bags until use.

Efficiency of probiotic microencapsulation

The microencapsulation efficiency of probiotic microorganisms (MEP) was evaluated using one gram of the suspension before drying or one gram of the microcapsules which were

mixed with 9 mL of sterile peptone water (0.1 %, w/v). Viable cell counts were determined in triplicate by plate seeding using MRS agar supplemented with L-cysteine (0.05 % w/v) and incubated at 37 °C (72 h). Previous results showed that the morphology of colonies was different for *Lactobacillus acidophilus* and *Bifidobacterium* spp. The results were expressed as a log CFU/g sample as suggested by Pupa *et al*. (2021). The MEP was calculated by equation1:

$$\text{MEP}\ (\%) = (\text{N}/\text{No}) \times\ 100 \quad (1)$$

where No and N represented the log of the number of viable cells (CFU) before and after the encapsulation process, respectively.

Powder properties

After simple and double spray drying, microcapsules were characterized in terms of water solubility index (WSI), water absorption rate (WAR), swelling capacity (SC), morphology, microencapsulation efficiency of phenolic compounds (MYp) and antioxidant activity (AA).

Water solubility index (WSI), water absorption rate (WAR) and swelling capacity (SC)

The WSI was determined according to Paini *et al*. (2015). One gram of the microcapsules was mixed with 12 mL of distilled water, mixed and incubated at 30 °C for 30 min. The sample was then centrifuged at 3500 rpm for 10 min. The supernatant was transferred to a capsule and dried at 105 °C until it reached a constant weight. The WSI, WAR, and SC were calculated using equations 2, 3, and 4, respectively:

$$\text{WSI}\ (\%) = \frac{\text{Supernatant dried weight}}{\text{Initial weight microcapsules}} \times 100 \quad (2)$$

$$\text{WAR}\ (\text{g/g}) = \frac{\text{Fresh sediment weight}}{\text{Initial weight microcapsules}} \quad (3)$$

$$\text{SC}\ (\text{g/g}) = \frac{\text{Supernatant dried weight}}{\text{Initial weight microcapsules}\ (100 - \text{WSI})} \quad (4)$$

Microcapsule morphology

The morphology of the microcapsules was examined by scanning electron microscopy (SEM) using a high-resolution, high-vacuum microscope (SM-71480 JEOL, Massachusetts, USA). The microcapsules were attached to the sample holder with double-sided adhesive tape. SEM images were taken at room temperature and examined using an acceleration voltage of 15 kV according Navarro-Flores *et al*. (2020).

Microencapsulation efficiency of phenolic compounds (MYp)

The microencapsulation efficiency of phenolic compounds (MYp) was calculated by using the total and superficial phenolic compounds in microcapsules, following the methodology described by Navarro-Flores *et al*. (2020). Briefly, to measure the total phenol content, 200 mg of the microcapsules were mixed with 2 mL of methanol:acetic acid:water solution (50:8:42 v/v/v). The mixture was shaken for 1 min, sonicated twice in a Cole-Palmer ultrasonic bath model 08855-00 (Cole-Palmer, Vernon Hills, IL, USA) at 25 °C for 20 min, and finally centrifuged at 4000 rpm for 5 min. The supernatant was used for quantifying the total phenolic content. For determination of superficial phenolic compounds content, 200 mg of the microcapsules were mixed with 2 mL of ethanol:methanol solution (1:1), agitated for 1 min, and then centrifuged at 4,000 rpm for 5 min, and the content of phenolic compounds was determined according to Navarro-Flores *et al*. (2020). The content of the total and superficial phenolic compounds was determined with Folin-Ciocalteu reagent, with the method described by Singleton *et al*. (1999) using gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of powder. The efficiency of the microencapsulation of phenolic compounds was determined by Eq. 5:

$$\text{MYp}\ (\%) = \frac{\text{PCtotal} - \text{PCsup}}{\text{PCtotal}} \times 100 \quad (5)$$

where PCtotal is the total phenolic compound (mg GAE/g) and PCsup is the superficial phenolic compound (mg GAE/g).

Antioxidant activity (AA)

The AA was determined by measuring the inhibitory effect against the DPPH radical, following the method described by Shekhar and Anju (2014), with some modifications. Briefly, several microcapsules´s solutions (25, 50, 100, 150, and 200 µg/mL) were prepared. Three milliliters of each solution were mixed with 1 mL of DPPH (0.1 mM). After 30 min of incubation, the absorbance of the solution was measured at 517 nm. The AA was calculated using equation 6:

$$\text{AA}\ (\%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (6)$$

where Abs control is the absorbance of the control and Abs sample is the absorbance of the sample.

The EC_{50} value of the sample, which represents concentration required to inhibit 50 % of the DPPH radical, was calculated using the inhibition curve.

Finally, microcapsules with the best properties, such as low solubility index, higher antioxidant activity, and greater encapsulation efficiency of phenolic compounds and probiotics, were selected for the next stage of this research. Once selected, the effect of applying these microcapsules to fresh pieces of jicama was studied.

Coating of minimally processed jicama roots

To determine the effect of the addition of the edible coating on the physicochemical and microbiological properties of the minimally processed jicama roots, five types of coatings were evaluated: (1) gelatin-maltodextrin (1:25) 26 % (w/w) aqueous solution (referred as "C"); (2) GCE at a final concentration of 4.2 mg GAE/mL and *Lactobacillus acidophilus* and *Bifidobacterium* spp. at a final concentration of 10^9 and 10^8 CFU/mL, respectively, added to a gelatin-maltodextrin (1:25) at 26

% (w/w) aqueous solution (referred as "EP"); (3) GCE at a final concentration of 4.2 mg GAE/mL added to gelatin-maltodextrin (1:25) 26 % (w/w) aqueous solution (referred as "E"); (4) *Lactobacillus acidophilus* and *Bifidobacterium* spp. at a final concentration of 10^9 and 10^8 CFU/mL, respectively, added to gelatin-maltodextrin (1:25) 26 % (w/w) aqueous solution (referred as "P"), and (5) microcapsules obtained after double spray drying process (referred as "MC").

The jicama roots were washed, disinfected, and cut into cubes (2 x 2 x 1 cm, 5±0.5 g) using a sterile knife. For coatings C, EP, E, and P, the jicama roots were immersed in the respective mixtures for one minute. For treatment MC, the jicama was coated with a thin layer of powder (approximately 0.3 grams of microcapsules per piece). Previous results indicated that this method produced a uniform layer of the microcapsules on the surface of each jicama piece. The samples coated were stored in polypropylene containers (4 x 6 x 6 cm) at 4 °C for six days. Microbiological and physicochemical properties (weight loss, pH, color, total phenolic compounds, caffeine and chlorogenic acid) of the samples were analyzed at 0, 1, 3, and 6 days.

Determination of shelf-life characteristics of jicama roots

To measure the weight loss, the jicama was weighed on the sampling days. The total weight loss was calculated by equation 7:

$$\text{Total weight loss (\%)} = \frac{\text{Weight (t)}}{\text{Weight on day 0}} \times 100 \quad (7)$$

The surface color of the jicama was determined using a portable colorimeter (ColorTec, Clinton, NJ, USA). The values of L* (luminosity), a* (-green a + red), and b* (-blue a + yellow) were recorded, and the chromaticity (C*) was then calculated using equation 8:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (8)$$

To measure the pH, the samples were blended, and the juices were centrifuged at 4500 rpm for 3 min at 4 °C before measuring pH using a pH meter (Hanna Instruments HI981031, Woonsocket, RI, USA).

Quantification of total phenolic compounds (TPC), caffeine (CF) and chlorogenic acid (CL) in the jicama roots

The extracts used for TPC, CF and CL determinations were obtained according to the methodology described by Desai *et al*. (2019). For that, jicama samples were freeze-dried using a lyophilizer (Labconco FreeZone 4.5 L, Kansas City, USA) at -40 °C and 0.250 mbar for 48 h. Then, 0.5 g of the lyophilized sample was mixed with 2.5 mL of a methanol:acetic acid:water solution (50:8:42 v/v/v). The mixture was shaken for 1 min, sonicated twice in a Cole-Palmer ultrasonic bath model 08855-00 (Cole-Palmer, Vernon Hills, IL, USA) at 25 °C for 20 min, and finally centrifuged at 4000 rpm for 5 min. The supernatant was used for quantifying the phenolic compound content according to Joya-Dávila *et al*. (2023).

Caffeine and chlorogenic acid were quantified by high-performance liquid chromatography (HPLC) with a Kromasil 100-5-C18 column (4.6 x 150 mm, 5 µm, 100 A-Supelco, Bellefonte, CA, USA), using a diode array detector (PerkinElmer Series 200 HPLC Systems, Shelton, CT, USA). The samples were filtered with a 0.22 µm millipore membrane. The mobile phase was acetonitrile/formic acid at 0.1 % (80:20, v:v) (Phase A) and formic acid at 1 % (v/v) (Phase B) at a 10:90 ratio with a constant flow of 1 mL per min in isocratic mode. Quantification was performed at 280 nm for CF and 320 nm for CL, and 10 µL of the sample were injected into the HPLC. In addition, standard solutions of the analytes to be quantified (50, 100, 200, 300, 400, 500, and 700 mg/L) were prepared for elution times and respective calibration curves. Metabolites were expressed in milligram GAE per gram of jicama root in dry basis.

Experimental design and statistical analysis

A completely randomized experimental design with three replicates was employed for two evaluations. The results were analyzed using an analysis of variance (ANOVA) to determine significant differences between treatments ($p \leq 0.05$). Honestly-significant-difference (HSD) or Tukey test were used for mean comparisons. Statistical analyses were carried out using Statgraphics Centurion XVI software.

RESULTS AND DISCUSSION

Survival of probiotics during the spray drying process

The results indicated that the encapsulation efficiency of *Lactobacillus acidophilus* and *Bifidobacterium* spp. was not significantly affected by the inlet air temperature, regardless of whether single or double spray drying was used. After the single drying, cell viability ranged from 9.39 to 9.12 log CFU/g for *Lactobacillus acidophilus* and 7.91 to 7.85 log CFU/g for *Bifidobacterium* spp. The encapsulation efficiency of both microorganisms at 120 or 140 °C after the single drying was around 90 % (Table 1). However, the encapsulation efficiency for both microorganisms after the double drying process was around 73- 77 %. Despite this reduction, cell viability remained at 7.2 log CFU/g for *Lactobacillus acidophilus* and 6.2 log CFU/g for *Bifidobacterium* spp. Despite the decrease in cell viability, the use of chitosan as a coating material allowed obtaining powders with a probiotic content higher than 10^6 CFU/g of microcapsules. Similar results were reported by Pupa *et al*. (2021) and Flores-Belmont *et al*. (2015), who encapsulated different species of lactic acid bacteria with chitosan through a double spray drying process, with encapsulation efficiencies of approximately 70 %.

Microcapsules properties

Micrographs, water solubility index (WSI), water absorption rate (WAR) and swelling capacity (SC) of microcapsules after single and double spray drying

Micrographs show that the microcapsules had a spherical

Table 1. Inlet air temperature effect on microencapsulation efficiencies of *Lactobacillus acidophilus* and *Bifidobacterium* spp. after the spray drying process.
Tabla 1. Efecto de la temperatura del aire de entrada sobre la eficiencia de microencapsulación de *Lactobacillus acidophilus* y *Bifidobacterium* spp. después del proceso de secado por aspersión.

Spray drying	Inlet air temperature (°C)	*Lactobacillus acidophilus* (%)	*Bifidobacterium* spp. (%)
Single	120	92.13±1.28 a*	92.55±1.34 a
	140	92.28±1.49 a	91.31±2.83 a
Double	120	76.24±0.54 b	77.18±0.11 b
	140	73.30±1.07 b	73.48±2.23 b
HSD		4.68	7.81

* Means followed by different lowercase letters in a column are significantly different according to the Tukey HSD test ($p \leq 0.05$).
Medias seguidas con diferentes letras minúsculas en una columna son significativamente diferentes de acuerdo a la prueba de Tukey ($p \leq 0.05$).

shape with dents and free of cracks on the surface, and an approximate average diameter of 15 µm (Figure 1). For microcapsules obtained by single and double spray drying at 120 and 140 °C, the size did not differ. The solubility of the microcapsules obtained by single spray drying ranged from 88 to 89 % (Table 2). These results are similar to those reported by Navarro-Flores *et al*. (2020), who encapsulated phenolic compounds using maltodextrin and other unconventional agents. These high solubility index could be attributed to the high solubility of the encapsulant agents (Fazaeli *et al*., 2012). Additionally, gelatin and maltodextrin contain hydrophilic sections, so they could interact and create a more soluble particle in aqueous environments (Semenova *et al*., 2002). Moreover, the water solubility index decreased for the microcapsules obtained by double microencapsulation through spray drying with chitosan (Table 2), compared with microcapsules obtained by single spray drying. This reduction can be attributed to the low solubility of chitosan at pH values above 6.5 (Aranaz *et al*., 2021). These results are similar to those reported by Flores-Belmont *et al*. (2015), who indicated that double microencapsulation with chitosan resulted in less insoluble powders in water (pH 7).

Water absorption rate values of microcapsules ranged from 0.11 to 0.34 g/g (Table 2). These values are similar to those reported by other authors (Da Costa *et al*., 2018; Navarro-Flores *et al*., 2020). It has been reported that variations in WAR may be due to the different degrees of participation of hydroxyl groups present in encapsulant agents in the formation of hydrogen bonds with water (Ahmed *et al*., 2010; Da Costa *et al*., 2018). The WAR of microcapsules obtained by single spray drying were lower than those by double spray drying with chitosan. This reduction can be attributed to the low solubility of chitosan at pH values above 6.5 as mentioned previously. At basic pH, the amino groups of chitosan are in their deprotonated form ($-NH_2$), which reduces their ability to interact with water and decreases the polymer´s solubility (Aranaz *et al*., 2021). SC values ranged from 0.037 to 0.092 g/g (Table 2). The SC values decreased significantly ($p \leq 0.05$) for the microcapsules obtained by double spray drying

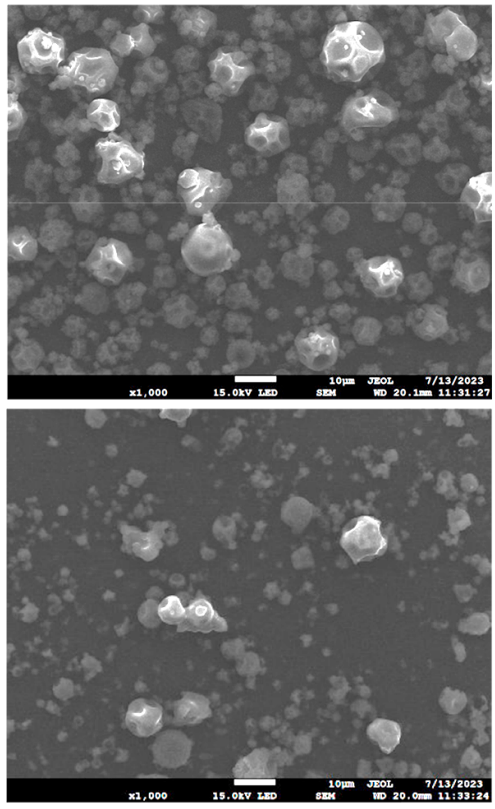

Figure 1. SEM micrographs of spray-dried powder particle; simple spray-dried process (a) and the double spray-dried process (b).
Figura 1. Micrografías SEM de partículas de polvo secado por aspersión; secado por aspersión simple (a) y secado por aspersión doble (b).

compared with microcapsules obtained by single spray drying, probably due to the presence of chitosan. Ahmed *et al*. (2010) reported that a low swelling capacity is related to the greater stability of microcapsules, which reduces their ability to swell.

Encapsulation efficiency of phenolic compounds and antioxidant activity of microcapsules after single and double spray drying

The inlet air temperature and the double encapsulation process with chitosan did not have a statistically significant effect ($p \geq 0.05$) on the encapsulation efficiency of phenolic compounds, with percentages ranges of 91.60 - 93.16 % (Table 3). The total phenol content in microcapsules ranged from 8.06 to 11.89 mg GAE/g of powder (Table 3). These values are similar to those reported by Desai *et al*. (2019) for green coffee extract encapsulated with maltodextrin, with a TPC of 11.98 mg GAE/g of powder.

The results indicated that increasing the inlet air temperature to 140 °C or the double spray drying process, the total phenol concentration of the microcapsules decreased significantly ($p \leq 0.05$). Despite the decrease in phenolic compounds, the antioxidant activity, measured as IC_{50}, remained unchanged. During spray drying, some phenolic compounds may degrade; however, new derivative compounds that are highly effective at inhibiting free radicals can be formed, as noted by Abrahão *et al*. (2019). Additionally, during thermal

Table 2. Water solubility index, water absorption, swelling capacity, phenolic compounds, chlorogenic acid content, caffeine content, IC_{50}, and the microencapsulation efficiency of phenolic compounds of microcapsules by simple and double spray drying.
Tabla 2. Índice de solubilidad en agua, absorción de agua, capacidad de hinchamiento, compuestos fenólicos, contenido de ácido clorogénico, contenido de cafeína, IC_{50} y eficiencia de microencapsulación de compuestos fenólicos de microcápsulas mediante secado por aspersión simple o doble.

Treatment	Water solubility index (%)	Water absorption rate (g/g)	Swelling capacity (g/g)	Phenolic compound (mg EAG/g)	Chlorogenic acid (mg/g)	Caffeine (mg/g)	Microencapsulation efficiency of phenolic compound (%)	IC_{50} (µg/mL)
Single 120 °C	88.53±1.47 ^{a*}	0.11±0.04 ^{b}	0.078±0.011 ^{a}	11.89±0.01 ^{a}	4.57±0.02 ^{a}	2.74±0.38 ^{a}	92.14±0.11 ^{a}	110.21±2.61 ^{a}
Single 140 °C	89.93±1.95 ^{a}	0.12±0.02 ^{b}	0.092±0.022 ^{a}	8.93±0.66 ^{b}	4.43±0.22 ^{a}	2.70±0.04 ^{a}	93.16±0.60 ^{a}	114.43±1.65 ^{a}
Double 120 °C	78.90±0.76 ^{b}	0.34±0.05 ^{a}	0.037±0.002 ^{b}	9.62±0.12 ^{b}	4.21±0.31 ^{a}	2.47±0.05 ^{a}	91.60±0.40 ^{a}	111.42±3.37 ^{a}
Double 140 °C	78.39±0.15 ^{b}	0.30±0.08 ^{a}	0.037±0.002 ^{b}	8.06±0.44 ^{b}	4.28±0.01 ^{a}	2.22±0.12 ^{a}	92.19±0.23 ^{a}	116.28±0.97 ^{a}
HSD	3.34	0.14	0.03	1.64	0.77	0.83	1.56	9.51

*Means followed by different lowercase letters in the same column are significantly different according to the Tukey HSD test ($p \leq 0.05$).
Medias seguidas con diferentes letras minúsculas en la misma columna son significativamente diferentes de acuerdo a la prueba de Tukey ($p \leq 0.05$).

Table 3. Total phenolic compounds content in jicama roots during storage at 4 °C for six days.
Tabla 3. Contenido total de compuestos fenólicos en jícama durante el almacenamiento a 4 °C durante seis días.

Phenolic compounds (mg GAE/g of jicama roots in dry base)					
Treatment	Time (days)				HSD
	0	1	3	6	
C	0.99±0.01 ^{cA*}	0.99±0.01 ^{cA}	0.96±0.01 ^{cA}	0.99d±0.02 ^{cA}	0.06
EP	1.50±0.02 ^{bA}	1.50±0.04 ^{bA}	1.50±0.01 ^{bA}	1.49.44±0.01 ^{bA}	0.10
E	1.50±02 ^{bA}	1.50±0.03 ^{bA}	1.50±0.03 ^{bA}	1.49±0.01 ^{bA}	0.07
P	0.97±0.01 ^{cA}	0.98±0.01 ^{cA}	0.98±0.1 ^{cA}	0.98±0.01 ^{cA}	0.03
MC	4.31±0.12 ^{aA}	3.93±0.02 ^{aAB}	3.38±0.21 ^{aBC}	3.00±0.12 ^{aC}	0.57
HSD	0.22	0.09	0.40	0.22	

C (Control), EP (green coffee extract/probiotics), E (green coffee extract), P (probiotics), MC (microcapsules with chitosan obtained by double spray drying). *Means followed by different lowercase letters in the same column are significantly different according to the Tukey HSD test ($p \leq 0.05$). Means followed by different uppercase letters in the same row are significantly different according to the Tukey HSD test ($p \leq 0.05$).
C (Control), EP (extracto de café verde/probiótico), E (extracto de café verde), P (probióticos), M (microcápsulas con quitosano obtenidas mediante doble secado por aspersión). * Medias seguidas con diferentes letras minúsculas en la misma columna son significativamente diferentes de acuerdo a la prueba de Tukey ($p \leq 0.05$). Medias seguidas con diferentes letras mayúsculas en la misma linea son significativamente diferentes de acuerdo a la prueba de Tukey ($p \leq 0.05$).

process, the Maillard reaction can occur, producing complexes with varying degrees of antioxidant activity (Liang *et al*., 2016).

The results also showed that around 110 - 116 µg/mL of microcapsules was needed to inhibit 50 % of DPPH radical (IC_{50}), and the double encapsulation process did not significantly affect this IC_{50} value. In addition, neither the inlet air temperature and double encapsulation caused significant differences in the concentrations of chlorogenic acid and caffeine. This suggests that the double encapsulation process using chitosan effectively preserves both phenolic compounds and their antioxidant activity.

Based on these results, only the microcapsules obtained by double spray drying with chitosan at an inlet air temperature of 120 °C were used for the subsequent jicama coating experiments.

Coating of minimally processed jicama roots

Determination of shelf-life characteristics

Weight loss results (Figure 2a) showed that jicama coated with microcapsules obtained by double encapsulation with chitosan (MC) showed the highest weight loss at the end of a 6 days of storage. Moreover, EP, E, and P treatments provided a significant additional protection ($p \geq 0.05$) against weight loss compared to treatment MC. Wong *et al.* (2021) reported that weight loss in food during storage is mainly due to water migration from plant tissues to the outdoor environment through transpiration. In addition, the moisture difference between the jicama and the environment was probably the driving force for weight loss.

The pH of the jicama decreased significantly during storage ($p \leq 0.05$), with the lowest pH values observed in treatments containing probiotic microorganisms (MC, EP and P) compared to the treatments without probiotics

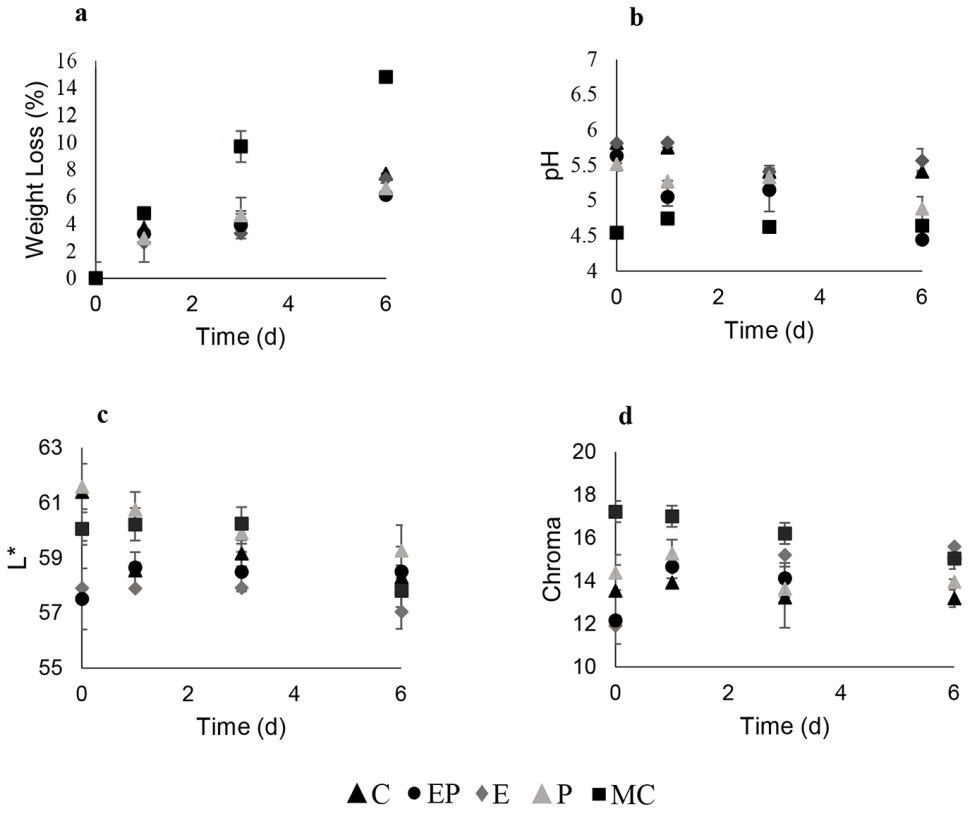

Figure 2. Effect of different types of coatings on weight loss (**a**), pH (**b**), luminosity (**c**) and chromaticity (**d**) of jicama during storage at 4 °C for six days. C (Control); EP (green coffee extract/probiotics); E (green coffee extract); P (probiotics); MC (microcapsules with chitosan obtained by double spray drying).
Figura 2. Efecto de diferentes tipos de recubrimientos sobre la pérdida de peso (**a**), pH (**b**), luminosidad (**c**) y cromaticidad (**d**) de jícama mínimamente procesada durante el almacenamiento a 4 °C durante seis días. C (Control); EP (extracto de café verde/probióticos); E (extracto de café verde); P (probióticos); MC (microcápsulas con quitosano obtenidas por secado por aspersión doble).

(Figure 2a). This could be attributed to the fact that during storage of jicama, even under refrigeration, probiotics could use the nutrients present in the coating and/or jicama pieces, producing organic acids, such as lactic acid, which could cause a decrease in pH. Wong *et al.* (2021) reported similar pH decreases in fresh-cut apple slices coated with *Lactobacillus plantarum*. The decrease in pH during the storage of minimally processed foods can be attributed to the activity of endogenous enzymes, which can produce acids and contribute to pH reduction. In treatments containing probiotics, the pH decrease can be attributed to the microbiological activity of the added microorganisms (Varoquaux and Wiley, 2017). Among the treatments with probiotics, the most pronounce pH reduction in jicama was observed in those with free microorganisms (treatments EP and P) (Figure 2a). This pH reduction could suggest a likely decrease in product acceptance. Otherwise, the pH of the jicama coated with the microcapsules (treatment MC) remained almost constant during storage. This could be attributed to the fact that spray drying decreased the cell metabolic activity of the bacterial cells (Behboudi-Jobbehdar *et al*., 2013).

Color is another important attribute of minimally processed foods, as affects the appearance and consumer's acceptance of the product. On the cutting surface, cell rupture can occur, allowing substrates and oxidizers to come into contact. Therefore, one of the main objectives during the minimum processing of fruit and vegetables is to preserve the original color. Changes in the color of the samples were expressed through chromaticity (value C*) and luminosity (L*) with respect to time (Figure 2 c). In general, the results indicated that at the beginning of storage, the samples that had the coating with the unencapsulated green coffee extract (E and EP) were significantly opaquer than the control, whereas samples coated with powder obtained from double spray drying showed L* values similar to the control treatment. This behavior could be attributed to the color masking effect of the encapsulant agents on the color of the green coffee extract (Piedrahíta *et al*., 2018). After 6 days of storage, however, there was no significant statistical difference ($p \geq 0.05$) in the luminosity of the samples. Color changes in the samples were expressed through chromaticity (C* value) over time; after 6 days of storage, there were no significant changes in the color of the samples.

Viability of probiotic microorganisms in jicama

The viability of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in different types of coatings for minimally processed of jicama´s pieces during storage at 4 °C are shown in Figures

3a and 3b, respectively. As can be seen, the number of *Lactobacillus acidophilus* and *Bifidobacterium* spp. cells remained constant during the 6 days of storage in EP and P treatments. This could have occurred because microorganisms remain metabolically active, probably by using impregnated carbohydrates and/or nutrients from the jicama as a carbon source (Wong *et al*., 2021). For the MC treatment (jicama coated with microcapsules) after 3 days of storage, the viability of microorganisms decreased significantly ($p \leq 0.05$) (Figures 3a and 3b), probably because double spray drying with chitosan caused damage to the cell membrane of the probiotics. Therefore, when the microcapsules were applied to the jicama, they were hydrated, and the probiotics were reactivated. However, the microorganisms, having been damaged in the double drying, began their death phase.

Total phenol, caffeine, and chlorogenic acid content during the storage of minimally processed jicama roots

The results of the total phenol content (mg/g of jicama roots in dry basis) during storage are presented in Table 3. The control treatment had a total phenol concentration of 0.99 mg GAE/g. Treatments E, EP, and MC exhibit higher total phenol content than the control (C). These results could be attributed to the green coffee extract present in these treatments, which were reported as an excellent source of phenolic compounds (Desai *et al*., 2019). The main phenolic compounds present in the green coffee extract are caffeine and chlorogenic acid, so these metabolites were quantified (Supplementary Fig. S1). Treatments C and P did not show detectable levels of caffeine or chlorogenic acid. In contrast, treatments E and EP resulted in caffeine and chlorogenic acid content to remain in the range of 0.43 - 0.38 mg/g and 0.42- 0.41 mg/g, respectively, throughout storage.

In the MC treatment, the content of caffeine (0.72 - 0.48 mg/g) and chlorogenic acid (1.11- 0.55 mg/g) were significantly higher ($p \leq 0.05$) during the entire storage compared with the other treatments. During storage, the concentration of total phenols, caffeine, and chlorogenic acid decreased

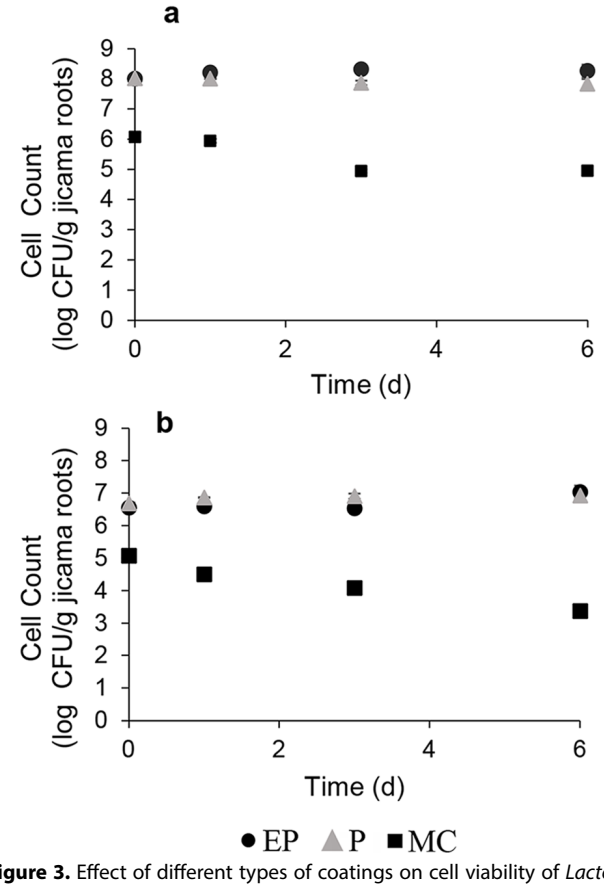

Figure 3. Effect of different types of coatings on cell viability of *Lactobacillus acidophilus* (**a**) and *Bifidobacterium* spp. (**b**) during the storage of jicama roots at 4 °C for six days. EP (green coffee extract/probiotics); P (probiotics); MC (microencapsulated with chitosan obtained by double spray drying). The other treatments are omitted because they were not inoculated.

Figura 3. Efecto de diferentes tipos de recubrimientos sobre la viabilidad celular de *Lactobacillus acidophilus* (a) y *Bifidobacterium* spp. (b) durante el almacenamiento de jícama a 4 °C durante seis días. EP (extracto de café verde/probióticos); P (probióticos); MC (microencapsulado con quitosano obtenido por secado por doble pulverización). Los demás tratamientos se omiten por no ser inoculados.

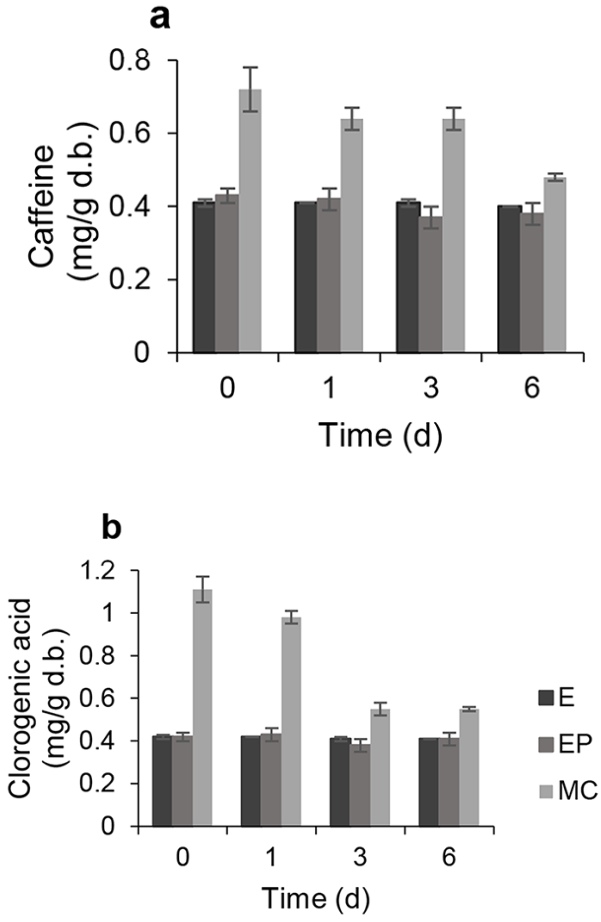

Figure S1. Effect of different types of coating on the caffeine (a) and chlorogenic acid (b) content in jicama roots during storage at 4 °C for six days. E: green coffee extract; EP: green coffee extract/probiotics; MC: Microencapsulated with chitosan obtained by double spray drying.

Figura S1. Efecto de diferentes tipos de recubrimiento sobre el contenido de cafeína (a) y ácido clorogénico (b) de raíces de jícama durante el almacenamiento a 4 °C durante seis días. E: extracto de café verde; EP: extracto de café verde/probióticos; MC: Microencapsulado con quitosano obtenido mediante secado por aspersión doble.

significantly ($p \leq 0.05$) in the samples of the MC treatment (jicama coated with the microcapsules obtained by double spray drying), compared with the other treatments. It has been reported that incorporating probiotics into vegetables matrices using the immersion technique allows microorganisms to enter the interior of the food through capillarity, promoting their adherence and protecting them from external conditions (De Oliveira *et al*., 2017). Additionally, refrigeration helps maintain the stability of probiotics, as reported by Wong *et al*. (2021). However, the MC treatment presents a higher content of these phenolic compounds (caffeine and chlorogenic acid) throughout the complete jicama storage. The reduction of these compounds concentration could be originated for the microcapsule's hydration and their partial hydration during the storage of the food, which causes the release of phenolic compounds into the outside environment. França *et al*. (2018) reported that in chitosan microcapsules the active compound is trapped in the nucleus and covered by a chitosan layer, which, depending on storage conditions, can swell and then release the nutrient.

CONCLUSIONS

In this study, microcapsules containing probiotics and phenolic compounds were obtained through single and double spray drying at 120 and 140 °C. Double spray drying with chitosan allowed microcapsules with a microbial population higher than 10^6 CFU/g and 9.62 mg GAE/g of phenolic compounds. Moreover, these microcapsules were used as an edible coating containing probiotics and phenolic compounds from green coffee, applied to minimally processed jicama root. Jicama pieces with encapsulated probiotics and phenolic compounds can be considered functional food due to their enhanced nutritional quality. To the best of the authors' knowledge, this is the first investigation incorporating a chitosan coating, obtained through a double spray drying process, into a minimally processed food. The microcapsules developed with the encapsulated probiotics and phenolic compounds allowed preserved the viability of probiotics and the concentration of phenolic compounds, such as caffeine and chlorogenic acid, in jicama during storage. This study reports the formulation and production of a functional food with high nutritional value and that can be used as a healthy snack. However, further research is recommended to extend the shelf life of coated jicama to enhance its appeal to industrial manufacturers.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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