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Artículo Científico

Encapsulation of betalains from beetroot stem by spray drying using *Aloe vera* gel as carrier

Encapsulación de betalaínas de tallo de betabel mediante secado por aspersión utilizando como agente acarrareador gel de *Aloe vera*

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Abstract

The effect of *Aloe vera* (*A. vera)* as a carrier on the encapsulation of beetroot stem juice by spray drying was studied. Mixtures in % A = *A. vera* and % B = beetroot stem juice were dried by spray: a) 25A:75B, 150 ºC, b) 25A:75B, 180 ºC, c) 75A:25B, 150 ºC, d) 75A:25B, 180 ºC. The obtained powders were evaluated for water activity (a_w), moisture (M), pH, glass transition temperature (T_g), bulk density (BD), water solubility index (WSI), color parameters, betacyanin (BC) and betaxanthin (BX) contents, antioxidant activity (AA) and morphology. The results show that with increasing *A. vera* H, pH, BD, T_g, WSI, BC, BX and the tendency to red decreased, while brightness and tendency to yellow increased and more compressed capsules were observed in the powders. Temperature affected WSI, color parameters a* and b* and individual betacyanins. The beetroot stem powders presented characteristics that allow stability: low a_w (<0.13), low moisture (<10 %), adequate pH for betalains (5.3-5.8) and T_g suitable for storage (around 50 ºC). The powder obtained with 25 % *A. vera* at 180 ºC presented a higher WSI and a tendency to red color, due to its higher BC and BX content, representing an alternative as a natural pigment in food formulation.

***Keywords*: betalains, spray drying, encapsulation, *Aloe vera*, beetroot stem**

Resumen

Se estudió el efecto del *Aloe vera* (*A. vera*) como acarreador en la encapsulación de jugo de tallo de betabel mediante secado por aspersión. Mezclas en % A = *A. vera* y % B = jugo de tallo de betabel fueron secadas por aspersión: a) 25A:75B, 150 ºC, b) 25A:75B, 180 ºC, c) 75A:25B, 150 ºC, d) 75A:25B, 180 ºC. Los polvos obtenidos se evaluaron para la actividad de agua (a_w), humedad (H), pH, temperatura de transición vítrea (T_g), densidad aparente (DA), índice de solubilidad en agua (ISA), parámetros de color, contenidos de betacianinas (BC) y betaxantinas (BX), actividad antioxidante (AA) y morfología. Los resultados muestran que el aumento de *A. vera* disminuye H, pH, BD, T_g, ISA, BC, BX y la tendencia al rojo, mientras que la luminosidad y la tendencia al amarillo aumentaron y se observaron cápsulas más comprimidas en los polvos. La temperatura afectó la ISA, los parámetros de color a* y b*, y las betacianinas individuales. Los polvos de tallo de betabel presentaron características que permiten la estabilidad: baja a_w (<0.13), baja humedad (<10 %), pH adecuado para betalaínas (5.3-5.8) y T_g apta para almacenamiento (alrededor de 50 ºC). El polvo obtenido con 25 % de *A. vera* a 180 ºC presentó un mayor ISA y tendencia al color rojo, debido a su mayor contenido BC, representando una alternativa como pigmento natural en la formulación de alimentos.

***Palabras clave*: betalaínas, secado por aspersión, encapsulación, *Aloe vera*, tallo de betabel**

1. Introduction

Beetroot (*Beta vulgaris*) has been catalogued among the ten important vegetables (Kavalcová *et al*., 2015). The intense red color of beetroot is due to high concentrations of betalains, and these are one of the compounds that provide beetroot with its antioxidant capacity (García *et al.,* 2016). Betalains from beetroot, are one of the most widely used natural colorants in the food industry as the red color is used in many food products. Of the beetroot plant, the main root or bulb is the part that is consumed; however, the stem contains betalains just like the bulb but it is not consumed directly, but can be considered as a by-product. The current trend is to recover valuable components of food by-products and recycle them within the food chain (Galanakis, 2015). The stem is a source of betalains (Slatnar *et al*., 2015; Aguirre-Calvo *et al*., 2018), with the potential to be used as a natural coloring alternative, the above with the purpose of formulating functional foods or to contribute to the new objectives in the food industry, such as obtaining clean label. Betalains from beet stems are a safe and healthy alternative of natural pigment, this approach as a current need.

Betalains are a group of water-soluble nitrogenous pigments with two structural groups: the red-violet group named betacyanins and the yellow-orange group named betaxanthins (Hussain *et al*., 2018). Betalains have also received much attention due to their benefits for human health, especially for their antioxidant and anti-inflammatory activities among others (García *et al.,* 2016); help in the treatment of some pathologies (Clifford *et al*., 2015). But despite its attractive color and health benefits there is a problem of stability of natural pigments when they are extracted from their sources, being highly sensitive to processing and storage conditions. To reduce the interactions of these compounds with factors to which they are sensitive, such as oxygen, humidity, light and the action of temperature and thereby increase their stability and shelf life, material packaging technologies can be used as a method of encapsulation. One technique for protecting and improving the stability of natural pigments is encapsulation by spray drying. This method has several advantages, including low cost and easy scalability. Even though spray drying has many advantages, the process is carried out at high temperature (>100 ºC) and it has been reported that temperature is one of the process conditions that can cause degradation of components such as betalains, specifically the betacyanins (Ruiz-Gutiérrez *et al*., 2014). A key point in the spray drying, to carry out the encapsulation it is necessary to use an adjuvant or carrier agent in the process. The most commonly used encapsulating agents are maltodextrin, gum arabic, and modified starch (Sánchez-Madrigal *et al*., 2019), but in the search for functional carriers, other types of compounds such as beta-glucans (Ruiz-Gutiérrez *et al*., 2014), inulin (Araujo-Díaz *et al*., 2017) and fructans (Sánchez-Madrigal *et al*., 2019) have been used, and more recently, matrices such as the use of maltodextrin-trehalose (Millinia *et al*., 2024), whey protein isolate (Wu *et al*., 2021) and ternary combinations of modified corn starch, gelatin, maltodextrin, tamarind gum, pectin, inulin and β-cyclodextrin (Guo *et al*., 2020) have been tested, to develop encapsulated powders with suitable properties for use as food ingredients.

A. vera gel is increasingly used in food products (Ray *et al*., 2013; Rodríguez-Rodríguez *et al*., 2020). Although the gel contains between 98.5 % and 99.5 % water, the solids are a combination of mucilage and other carbohydrates, organic acids and salts, enzymes, saponins, tannins, phenolic anthraquinones, flavonoids, flavanols, sterols, triacylglycerides, amino acids, vitamins, and minerals (Rodríguez-Rodríguez *et al*., 2020). This rich composition of functional compounds gives the gel a wide variety of biological activities, a bioactivity that can be used in ingredients for food formulation.

The objective of this study was to evaluate the use of *A. vera* gel as a carrier agent for encapsulation by spray drying of beetroot stem juice, and to study the physicochemical properties of the encapsulated powder obtained at different temperatures.

2. Materials and Methods

2.1 Materials

Beetroot Stem Juice. Whole beetroot plant (*Beta vulgaris*) was purchased from a local market in Chihuahua, Mexico. The stems were removed from the bulbs, then the stems were washed and disinfected [chlorine solution, 150 ppm] and processed through a domestic juice extractor. The juice obtained was vacuum filtered by passing the juice through of filter paper (No. 1; Whatman, Kent, UK). The juice was analyzed for moisture, pH, water activity, the total soluble solids (ºBx), antioxidant activity, and betalains (betacyanins and betaxanthins) content. The filtered juice was frozen and stored at −20 ºC in the dark until processing.

***Aloe vera* gel.** *A. vera* (*Aloe barbadensis Miller*) was purchased from the greenhouse of the Faculty of Chemical Sciences of the Autonomous University of Chihuahua. The *A. vera* leaves were selected for their size (40 to 50 cm), light gray green color and freshness. The leaves were washed and disinfected [chlorine solution, 150 ppm]. To obtain the gel, the acibar was first extracted by cutting the bases of the leaves and allowing them to drain vertically for one hour. Then, the epidermis was removed and the pulp obtained from *A. vera* gel was rinsed with distilled water to remove the residue of the acibar on the surface, processed with a domestic blender and the juice obtained was used immediately. Some characteristics such as pH, soluble solids, color parameters and antioxidant activity were determined.

2.2 Methods

2.2.1 Encapsulation of beetroot stem juice

Mixture: Mixtures of 450 mL of *A. vera* (% A) and beet stem juice (% B) were prepared 25A:75B and 75A:25B. The beetroot stem juice was prepared with maltodextrin [30 % (w/w, based on total soluble solids)] to increase the yield of the encapsulated powder obtained, due to the high-water content of the *A. vera* gel. Each mixture was replicated and was passed through a spray dryer keep with constant agitation.

Spray Drying: A spray dryer (Niro Mobile Minor DK-2860; GEA Company) with a rotary atomizer at 30 psi air pressure was used. The mixtures were fed through a peristaltic pump (Watson Marlow 504-U; Falmouth, Cornwall, UK) and dried at 150 and 180 ºC for the inlet temperature and 70 and 85 ºC for the outlet temperature, respectively. Spray drying process was performed in duplicate.

2.2.2 Analyses methods:

pH: pH was determined using a pH meter (HANNA Instruments, model EDFE HI2020, RI, USA) calibrated with standard pH 7.0 and 4.0 buffers. For encapsulated powders, the pH was determined using the method described by Kha *et al*. (2010). Encapsulated powder (5 g) was mixed in 25 mL of deionized water at 20 ºC. The determinations were performed in triplicate and the mean values with standard deviation were reported.

Total soluble solids: Total soluble solids were measured with the Abbe refractometer (Atago Co. Ltd., Tokyo, Japan) which was previously calibrated with sucrose solutions. The determinations were performed in triplicate and the mean values with standard deviation were reported.

Color: The color parameters of the luminosity (L*), green/red (-/+a*) and blue/yellow (-/+b*) were recorded samples were performed using Konica Minolta CR-400/410 colorimeter, calibrated with standards with values X= 94.9, y= 0.3185 and x= 0.3124. The determinations were made in triplicate and the mean values with standard deviation were reported.

Moisture: The moisture content was determined by gravimetric method 950.02 (AOAC., 1998). The analysis was carried out in triplicate and the mean values with standard deviation were reported. The results were expressed as g of water/100 g of encapsulated powder.

Water activity (a_w): The a_w was measured using a water activity meter (AquaLab Series 3 Quick Start; Decagon Devices Inc., Pullman, WA, USA). The analysis was performed in triplicate and the mean values with standard deviation were reported.

Bulk density (BD): The BD was determined using the method described by Kha *et al*. (2010). Encapsulated powder (2 g) was placed in a 10 mL graduated cylinder and the cylinder was held in a vortex vibrator (Vortex-1; Scientific Industries Inc., Bohemia, NY, USA) for 1 min. The BD value was calculated as the ratio of the mass in the cylinder to the volume occupied in the cylinder. The analysis was performed in triplicate and the mean values with standard deviation were reported.

Morphology. Capsules were observed at ×750 magnification with a scanning electron microscope (JSM-5800 LV; JEOL, Tokyo, Japan). The encapsulated powder was fixed on pieces of double-sided tape and coated with a thin layer of gold (-110 Å) using a sputter coating vacuum (Desk II; Denton vacuum LLC., Moorestown, NJ, USA). The samples were then examined by SEM at 10 kV.

Water Solubility Index (WSI). The WSI was determined according to Anderson *et al*. (1969). Encapsulated powder (2.5 g) and 30 mL of deionized water were mixed vigorously using a vortexer (Vortex-1; Scientific Industries Inc.) and incubated in a 37 ºC water bath for 30 min. The mixtures were centrifuged at 3600 x g for 60 min (Centra Centrifuge CL3R IEC; Thermo Electron Corporation, Waltham, MA, USA). The supernatant was carefully collected in trays previously weighed to constant weight and dried (Shel Lab; Sheldon Manufacturing, Inc., Cornelius, OR, USA) at 70 ºC for 36 h. The WSI was calculated as the percentage of the weight of the dried supernatant compared to the pre-dried weight of the powder. The analysis was performed in triplicate and the mean values with standard deviation were reported.

Glass transition temperature (T_g): The T_g was measured according to the described method by Ahmed *et al*. (2010) using a differential scanning calorimeter (TA Q-200; TA Instruments, Crawley, UK). Encapsulated powder (8 mg) was placed in an aluminum dish and sealed. An empty sealed capsule was used as a reference. The temperature scanning program changed a range of 70 - 120 ºC, followed by cooling to 30 ºC at a temperature ramp of 10 ºC/min under a nitrogen gas atmosphere. Thermograms were analyzed using universal analysis software (TA Instruments). The glass transition temperature was interpreted as the midpoint of the curves obtained. The analysis was performed in duplicate and the mean values with standard deviation were reported.

Betalains (BT) content: Both pigments, betacyanins and betaxanthins contents, were determined by spectrophotometric method. Beetroot stem juice was diluted with distilled water and the measurement was performed at a wavelength of 535 and 483 nm. The contents were expressed in mg/L of betacyanins and betaxanthins, respectively; using the following equation (Castellar *et al*., 2003):

$$\boldsymbol{BT\ (mg/L) = \frac{(A)(DF)(Mw)(1000)}{(\varepsilon)(L)}(100)} \qquad \textbf{Ec. (1)}$$

For encapsulated powders, the pigments were extracted according to the method of Castellanos-Santiago and Yahia (2008) with some modifications. Encapsulated powders (100 mg) were stirred in 10 mL of deionized water for 15 s using an Ultra-Turrax IKA T18 basic with an S18N19G homogenizer-dispersing tool (IKA Works Inc.). The samples were then centrifuged at 3,600 × g at 10 ºC for 40 min (Centra centrifuge CL3R IEC; Thermo Electron Corporation). This procedure was repeated to ensure efficient extraction. The supernatants were filtered through a 0.45 μm nylon filter (Millipore Corp.). Extractions were performed in triplicate and analyzed by spectrophotometry (Perkin-Elmer) and identification and degradation (%) by HPLC (1200 series; Agilent Technologies). The photometric quantification of betalains (betacyanins and betaxanthins contents) were performed according to the method of Castellanos-Santiago and Yahia (2008) using a Lambda 25 UV-Vis spectrophotometer (Perkin Elmer). The betacyanin (BC) content and betaxanthin (BX) contents were calculated using the following equation:

$$\boldsymbol{BT(mg/g)} = \frac{[(\boldsymbol{A})(\boldsymbol{DF})(\boldsymbol{Mw})(\boldsymbol{Vd})]}{(\boldsymbol{\varepsilon})(\boldsymbol{L})(\boldsymbol{Wd})} \qquad \textbf{Ec. (4)}$$

where: A is the absorbance value at the absorption maxima of 535 and 483 nm for betacyanins and betaxanthins, respectively, DF is the dilution factor, Vd is the volume of the dry pulp solution (ml), Wd is the weight of the dry pulp (g), and L is the path length (1 cm) of the tray. The molecular weights (Mw) and molar extinction coefficients (ε) of betanin [Mw=550 g/mol; ε= 60,000 L/(mol cm) in water at 536 nm] and indicaxanthin I [Mw= 308g/mol; ε= 48,000L/(mol cm) in water at 481 nm] were used for the quantification of betacyanins and betaxanthins. Measurements were performed in triplicate and the mean values with standard deviation were reported.

Individual betacyanins were analyzed by high pressure liquid chromatography using the method described by Castellanos and Yahia (2008). Briefly, samples were filtered through 0.45 μm nylon filters, 20 μL were injected into a Thermo Scientific Dionex Ultimate 3000 UHPLC equipped with a Thermo Scientific reversed-phase column (C18, 250 x 4.6 mm; drop size 0.5 μm), and a UV detector (DAD Varian ProStar model 410, Palo Alto, CA). The mobile phase A: methanol/KH_2PO_4 0.05 M (18:82 v/v) pH=2.75 with phosphoric acid, and the mobile phase B: methanol, were adjusted to a gradient of 100 % A to 80 % A and 20 % B in 20 min at a flow rate of 1 mL/min. Detection was performed at 536 nm for betacyanins.

Antioxidant Activity (AA): The AA was determined according to the spectrophotometric method according to Kuskoski *et al*. (2005), based on the measurement of DPPH$^{\bullet}$ radical absorbance. First, 0.1 mL of extract was added to 3.9 mL of a DPPH$^{\bullet}$ radical solution (100 μM), vortexed (Vortex-1, Genie; Scientific Industries Inc.), and stored in the dark for 3 h. The absorbance was then measured at 517 nm using a Lambda 25 UV-Vis spectrophotometer (Perkin Elmer). A Trolox (0.08-1.28 mM) calibration curve was used. For juice the method described by Ruiz-Gutiérrez *et al*. (2014) was used. For encapsulated powders, the extracts were obtained according to the method described by Pitalua *et al*. (2010) with modifications; encapsulated powder (2.5 g) was dispersed in 50 mL of methanol and deionized water (1:1 v/v). The dispersions were homogenized for 15 s using an Ultra-Turrax (basic IKA T18) with a dispersing tool (S18N19G; IKA Works Inc.). The mixture was centrifuged at 3000 xg for 10 min (Centra centrifuge CL3R IEC; Thermo Electron Corporation). To ensure complete extraction, the process was repeated using 25 mL of methanol and deionized water (1:1 v/v). The supernatants were collected, filtered through a 0.22 μm pore polyethylene filter (Millipore Corp., Bedford, MA, USA). Results were expressed as μmol Trolox equivalents per gram of sample on a dry basis (mg TE/g). The determinations were performed in triplicate and the mean values were expressed as the standard deviation.

2.3 Experimental design and statistical analyses

A 2×2 completely randomized factorial design in duplicate was applied. The independent variables were carrier agent (25 and 75 %) and the inlet drying temperature (150 and 180 ºC). The dependent variables were the physical and chemical properties of the encapsulated powder. Data analysis was performed using Minitab (2018) software. The effect of the factors was determined using a two-way analysis of variance (ANOVA), and the Comparisons of means were calculated using Tukey's test at a significance level of 95 %.

3. Results y discussions

3.1 Beetroot stem juice characterization

Table 1 shows the characteristics of beetroot stem juice, the juice presented a pH of 5.98, a low soluble solids content (3.1 ºBrix). The color presented a low brightness and tendencies to redness and blueness due to the presence of betalains and chlorophyll, respectively. The chemical characteristics showed a total betalains content of 107.85 mg/kg, divided in 71.78 for betacyanins and 36.07 mg/kg of betaxanthins, showing that the main betalains group is betacyanins (red-purple color), and an antioxidant activity of 16.52 ± 0.74 (mmol TE/100g). Beetroot stem juice presents similar characteristics of pH, color and total betalains content to beetroot juice with values of pH = 6.5, 5.62, 2.13, -1.26 for L, a and b and 135.75 mg betalains/kg beetroot, according to Pitalua *et al*. (2010), the main difference is in soluble solids which is almost 4 times lower than beetroot juice.

Table 1. Characteristics* of beetroot steam juice and *A. vera* gel
Tabla 1. Características* de jugo de tallo de betabel y gel de *A. vera*

Determination	Juice	*A. vera*
pH	5.98 ± 0.1	4.66 ± 0.02
Soluble solids	3.1 ± 0.1	0.87 ± 0.02
Luminosity	23.8 ± 0.02	36.15 ± 0.1
Green-red tendency	2.75 ± 0.03	0.54 ± 0.01
Blue-yellow tendency	-2.37 ± 0.03	2.05 ± 0.05
Antioxidant activity (mmol TE/100g)	16.52 ± 0.74	6.71± 0.22
Betacyanins (mg/kg)	71.78 ± 1.88	-
Betaxanthins (mg/kg)	36.06 ± 1.24	-

For *A. vera* gel the pH was 4.66 and soluble solids of 0.87 ºBrix, results according to IASC (International Aloe Science Council), low soluble solids and acid pH. The color presented a slight tendency to yellow and a value of 6.72± 0.23 (mmol TE/100g) for antioxidant activity.

3.3 Encapsulated powders of beetroot stem juice

Four different powders were obtained and are showed in the Fig. 1, corresponding to the combinations of low content of *A. vera* in the mixture 25A:75B and high content of *A. vera* in the mixture 75A:25B at low spray drying temperature (150 ºC) and high spray drying temperature (180 ºC).

a) b) c) d)

Figure 1. Encapsulated powder of beetroot stem using different content of *A. vera* as carrier and dried at different temperatures: % A = *A. vera* gel and % B = beet stem juice; a) 25A:75B, 150 ºC; b) 25A:75B, 180 ºC; c) 75A:25B, 150 ºC; d) 75A:25B, 180 ºC.
Figura 1. polvo encapsulado de tallo de betabel secado a diferentes temperaturas utilizando *A. vera* como acarreador: % A = Gel de *A. vera* y % B = jugo de tallo de betabel; a) 25A:75B, 150 ºC; b) 25A:75B, 180 ºC; c) 75A:25B, 150 ºC; d) 75A:25B, 180 ºC.

In general, the powders obtained with low *A. vera* (25 %) show a more red-purple color, due to the higher content of betalains in the mixture, but the effect of the drying temperature is visible. The powder dried at higher temperature (180 ºC) showed a red-purple color more intense than the powder dried at lower temperature (150 ºC). On the other hand, the color in the powders obtained with high content of *A. vera* in the mixture (75 %) at both drying temperatures is very similar the color, then, it can be related to the reduction of the effect of drying temperature.

The analysis of the stability characteristics of the encapsulated powder of beetroot steam using *A. vera* as a carrier is presented in Table 2. The powders of the four treatments had a low a_w value, around to 0.1, this value ensures the stability of the powder and no differences were found between treatments. Some studies reported a low a_w values for powders obtained by spray drying as Ruiz-Gutiérrez *et al*. (2014) reported a_w values in red cactus pear encapsulated with soluble fiber as carrier (0.097 - 0.138) and Muñoz (2014) in beetroot juice dried using maltodextrin and grenetin as carriers (0.019 - 0.029).

Table 2. Characteristics* of encapsulated powder of beetroot steam by spray drying using *A. vera* as carrier
Tabla 2. Características* de polvo encapsulado de tallo de betabel secado por aspersión utilizando *A. vera* como acarreador

Temperature (ºC)	Mixture (A-B)	Moisture (%)	a_w	pH
150ºC	25:75	9.786 ± 0.038^a	0.127 ± 0.001^a	5.86 ± 0.07^a
180ºC	25:75	9.279 ± 0.419^a	0.118 ± 0.012^a	5.88 ± 0.02^a
150ºC	75:25	7.154 ± 0.101^b	0.130 ± 0.001^a	5.37 ± 0.03^b
180ºC	75:25	7.079 ± 0.026^b	0.120 ± 0.007^a	5.47 ± 0.05^b

*Mean ± standard deviation. A (%) = *A. vera*; B (%) = beetroot steam juice. Means with different letter by characteristic indicate significative ($p<0.05$) difference, by Tukey's test.

The moisture contents are low, values ranging from 7.079 ± 0.026 to 9.786 ± 0.038 and the pH values ranged from 5.37 ± 0.03 to 6.88 ± 0.02, for both variables the significant effect ($p<0.05$) of the *A. vera* content as a carrier in the mixture is observed. The encapsulated powders with 25 % of *A. vera* present higher moisture and pH values than the encapsulated powders with 75 % of *A. vera*, because *A. vera* is a gel and by adding more in the mixture there is a high overall moisture content that is dried under the same temperature condition. Moisture contents are similar to curcumin powders (1.9 % to 10.1 %), slightly higher than whey protein isolate powders for blackcurrant concentrate and higher than maltodextrin and trehalose matrices used for roselle anthocyanins, reported by Guo *et al*. (2020), Wu *et al*. (2021) and Millinia *et al*. (2024). Moisture values tend to decrease with increasing amount of carrier used. The pH values are suitable for the stability of the pigments, betalains are generally stable in the pH range of 4–6 (Martins *et al*., 2017), the pH values are statistically ($p<0.05$) lowest in powders with a 75 % *A. vera* content (75 %) due to the low pH of the gel.

Other important properties in the encapsulated powders are bulk density, glass transition temperature and water solubility index, these properties are important in relation to the behavior of the encapsulated powder during transportation, storage and application and are presented in Table 3.

Table 3. Properties* of encapsulated powder of beetroot steam by spray drying using *A. vera* as carrier
Tabla 3. Propiedades* de polvo encapsulado de tallo de betabel secado por aspersión utilizando *A. vera* como acarreador

Temperature (ºC)	Mixture (A-B)	Bulk density (g/mL)	T_g (ºC)	WSI
150ºC	25:75	0.730 ± 0.028[ab]	55.98 ± 2.81[a]	89.49 ± 0.23[a]
180ºC	25:75	0.755 ± 0.007[a]	53.80 ± 4.22[a]	86.60 ± 0.39[b]
150ºC	75:25	0.680 ± 0.000[b]	48.36 ± 2.90[a]	81.84 ± 1.30[c]
180ºC	75:25	0.670 ± 0.014[b]	51.60 ± 0.91[a]	86.89 ± 0.08[ab]

*Mean ± standard deviation. A (%) = *A. vera*; B (%) = beetroot steam juice; T_g = Glass transition temperature; WSI = Water solubility index. Means with different letter by properties indicate significative ($p<0.05$) difference, by Tukey's test.

The bulk density ranges from 0.670 ± 0.014 to 0.755 ± 0.007 g/mL, although the values of the different treatments are close, there is a significant ($p<0.05$) effect of both the amount of *A. vera* used and the interaction of this factor with temperature, being the effect of the amount of *A. vera* being more important. The encapsulated powders produced with 75 % of *A. vera* have a lower density than the encapsulated powders produced with 25 % of *A. vera*, because the solids content in the *A. vera* gel is lower than the solids content in the beetroot stem juice. Bulk densities slightly lower than the present study have been reported, 0.497–0.525 g/mL for *Amaranthus* extract (Tabio-García *et al*., 2022) using Opuntia ficus-indica mucilage and maltodextrin as a carrier.

The T_g was statistically ($p>0.05$) equal among the four encapsulated powders, values ranging from 48.36 ± 2.90 to 55.98 ± 2.81 ºC. This temperature indicates the temperature at which the product changes its density, hardness, and stiffness and then retains its properties until reaching a temperature of about 50 ºC is reached. This T_g value is better than that reported by Ruiz-Gutiérrez *et al*. (2014) for encapsulated powders prepared by spray drying but using soluble fiber as a carrier, who reported values from 32.27 to 37.69 ºC, and similar to the T_g (43.74-49.65 ºC) reported for powders dried at 150 ºC using maltodextrin and Opuntia ficus-indica mucilage as a carrier (Tabio-García *et al*., 2022).

The water solubility index (WSI) was significantly ($p<0.05$) affected by the amount of *A. vera* used in the mixture (0.002) and the interaction of drying temperature - amount of *A. vera* (0.001), the values ranged from 81.84 ± 1.30 to 89.49 ± 0.23, respectively, these values were high compared to the WSI reported by Ruiz-Gutiérrez *et al*. (2014), who reported WSI values of 0.64 to 0.764 for red cactus pear juice dried with soluble fiber, and similar to Tabio-García *et al*. (2022), who reported 82.96–92.81 % using maltodextrin and *Opuntia ficus-indica* mucilage as a carrier for encapsulation of *Amaranthus* extract. Although the WSI values are similar among the powders, the encapsulated powder with 75 % of *A. vera* dried at 150 ºC presented the lowest WSI, and the encapsulated powder with 25 % of *A. vera* dried at 180 ºC presented the highest WSI.

The analysis of the morphology of the powders, obtained by scanning electron microscopy (SEM) is shown in Fig. 2, the images showed a matrix in the form of spherical capsules with little agglomeration. Fig. 2a and 2b correspond to encapsulated powders obtained with 25 % of *A. vera* and show more spherical and less compressed capsules compared to encapsulated powders obtained with 75 % of *A. vera* (Fig. 2c and 2d). This compression characteristic is normal in spray drying as it can occur at the time of elimination of moisture. The collapse of the wall is more noticeable in Fig. 2c and 2d, where *A. vera* predominates in the mixture, since it contains a higher moisture content than the beetroot stem juice, causing a more compressed sphere. Therefore, the removal of water deforms the spherical particle in the case of the two powders. Different matrices produce different coating wall densities, and consequently, different shapes and textures, the maltodextrin-trehalose matrix, as a carrier, achieved more spherical shapes and smoother surfaces than in the present study, due to the presence of trehalose sugar (Millian *et al*., 2024) or drying at a lower inlet temperature (120 ºC). Powders obtained at similar drying temperatures show similar shapes in the capsules of the powders obtained with a 25 % of *A. vera* of the present study (Ruiz-Gutiérrez *et al*. 2014).

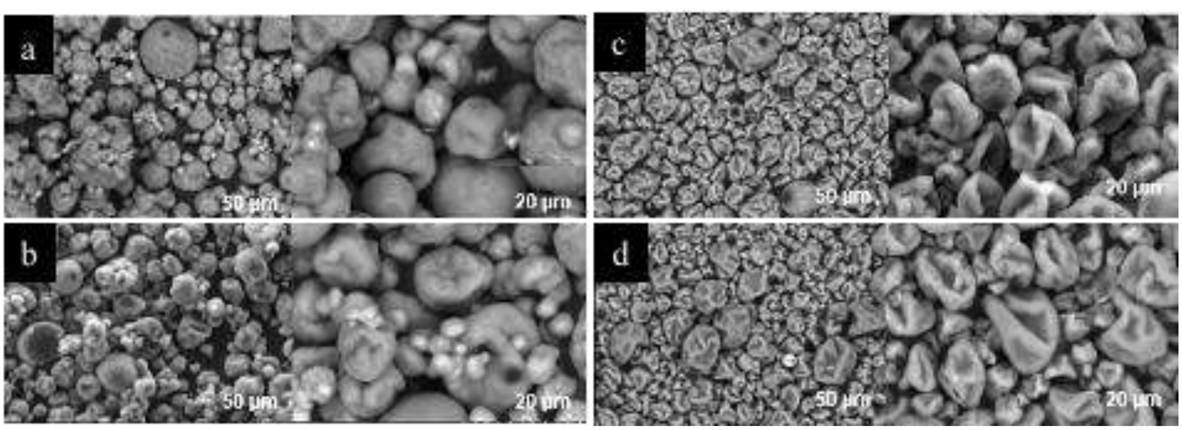

Figure 2. Encapsulated powder of beetroot stem with different content of *A. vera* as a carrier and dried at different temperatures; a) 25 % *A. vera* at 150 ºC, b) 25 % *A. vera* at 180 ºC, c) 75 % *A. vera* at 150 ºC, d) 75 % *A. vera* at 180 ºC.

Figura 2. Polvo encapsulado de tallo de betabel secado a diferentes temperaturas utilizando *A. vera* como acarreador; a) 25 % *A. vera* a 150 ºC, b) 25 % *A. vera* a 180 ºC, c) 75 % *A. vera* a 150 ºC, d) 75 % *A. vera* a 180 ºC.

However, in this case, the temperature does not affect either of the two powders, as these are very high temperatures that are capable of eliminating most of the water in the encapsulates.

The color parameters (L*, a* and b*) are presented in Table 4. The luminosity (L* parameter) presents values ranging from 26.74 to 53.26, L* was significantly ($p<0.05$) affected by percent of *A. vera* used in the mixture, finding that the encapsulated powder produced with 25 % of *A. vera* tends to dark color while that the encapsulated powder with 75 % of *A. vera* tends to white. Powders of whey protein isolate and black currant concentrate by spray drying showed a similar L* parameter value, 52.81 ± 0.50, (Wu *et al*., 2021) to powders with 75 % *A. vera* content.

Table 4. Color parameters* of encapsulated powder of beetroot steam by spray drying using *A. vera* as carrier dried at different temperatures.
Tabla 4. Parámetros de color de polvo encapsulado de tallo de betabel secado por aspersión utilizando *A. vera* como acarreador.

Temperature (ºC)	Mixture (A-B)	L*	a*	b*
150	25:75	26.74 ± 0.08[b]	15.91 ± 0.85[b]	-1.00 ± 0.03[b]
180	25:75	32.99 ± 5.62[b]	22.43 ± 1.08[a]	-1.54 ± 0.02[c]
150	75:25	53.26 ± 0.42[a]	8.44 ± 0.01[c]	13.88 ± 0.07[a]
180	75:25	51.32 ± 0.68[a]	8.07 ± 0.01[c]	13.96 ± 0.12[a]

*Mean ± standard deviation. A (%) = *A. vera*; B (%) = beetroot steam juice. Means with different letter by color parameter indicate significative ($p<0.05$) difference, by Tukey's test.

For the a* parameter, the significant effects were due to the *A. vera* used in the mixture and the drying temperature, showing more tendency to red color the encapsulated powder prepared with 25 % of *A. vera*, values were 15.91 ± 0.85 and 22.43 ± 1.08 at 150 and 180 ºC of drying temperature, respectively. For the encapsulated powder with 75 % of *A. vera* the values of parameter a*, for both temperatures, were around 8, being significantly ($p<0.05$) lower than the encapsulated powder elaborated with 25 % of *A. vera*. The difference is that this powder has less beetroot stem juice in the mixture, then less betalains, reducing the red tendency. For the b* parameter the effects and differences are the same as for the a* parameter, but the high b* values (around 13) were for encapsulated powders with 75 % of *A. vera*, indicating a tendency to yellow. The low b* values were for encapsulated powders with 25 % of *A. vera*, indicating a tendency to blue, and the powder dried at 180 ºC presents the tendency to blue (-1.54 ± 0.02). At high drying temperatures, there may be greater degradation of betaxanthins (yellow-orange pigments), reducing the tendency to +b* (yellow).

The betalains analyses showed the expected trend, the betalains content is shown in Fig. 3. The encapsulated powders with 25 % *A. vera* had a higher betalains content, because the mixture contained more beetroot stem juice. No significant differences ($p>0.05$) were observed by drying temperature. However, contrary to other studies, in which an increase in spray drying temperature was found to lead to a decrease in betacyanin content, however, the temperatures at which this effect was observed were > 180 ºC (Ruiz-Gutiérrez *et al*., 2014).

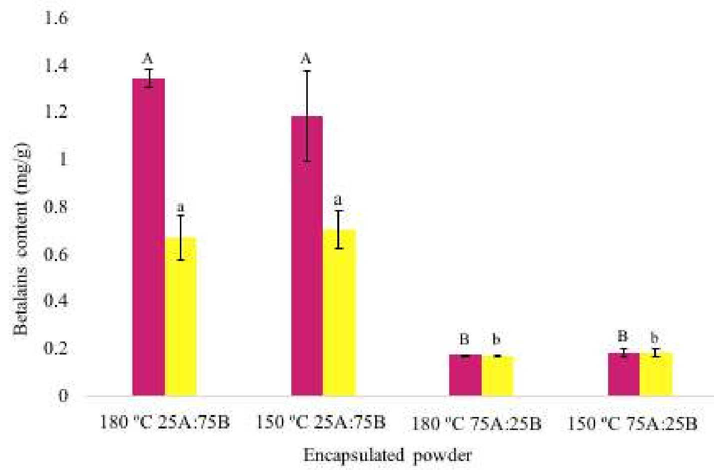

Figure 3. Betalains content on encapsulated powder of beetroot stem using different contents of *A. vera* as carrier and dried at different temperatures. ■ Betacyanins and ■ Betaxanthins. Mean ± standard deviation. A = *A. vera*; B = beetroot steam juice. Means with different letter by betalain group indicate significant ($p<0.05$) difference, by Tukey's test.

Figura 3. Contenido de betalainas de polvo encapsulado de tallo de betabel utilizando diferentes contenidos de *A. vera* como acarreador a diferentes temperaturas de secado. ■ Betacianinas y ■ Betaxantinas. Media con diferente letra por grupo de betalaína indican diferencia significativa ($p<0.05$), mediante prueba de Tukey.

Highlighting that in the encapsulated powders obtained with 25% *A. vera* in the mixture, the betacyanin content is about double compared to betaxanthins, being visually noticeable (Figs. 1a and 1b), presenting contents ranging from 1.185 to 1.343 mg/g and from 0.671 to 0.705 mg/g, for betacyanins and betaxanthins, respectively. However, encapsulated powders obtained with 75 % *A. vera*, the contents of betacyanins and betaxanthins do not present differences ($p>0.05$) showing contents around 0.35 mg/g of total betalains. These powders show a low tendency to red color and high tendency to yellow color (Table 3), and visually they have a pale beige color (Figs. 1c and 1d). The total betalains content is similar to that reported by Ruiz-Gutiérrez *et al*. (2014) for powder of red cactus pear powder by spray-drying using soluble fiber as a carrier, with values around of 1.4 – 1.8 mg/g for betacyanins and around 0.65 mg/g for betaxanthins were reported.

The analysis of betalains by HPLC helps to identify the individual betalains, especially the betalain that represents the group (betacyanins or betaxanthins) and which depends on the source, using the information reported by Slatnar *et al*. (2015) and Mikołajczyk-Bator & Pawlak (2016), such as retention times and elution order. Betacyanins are the most important group of pigments or with the highest content in the beetroot stem as shown by the quantification of total betalains carried out by UV-Vis spectroscopy (Fig. 3). These are the red-purple pigments and are quantified at a length of about 536 nm. The main individual pigments in this group are betanin, isobetanin, and neobetanin, although many more betacyanins may be present, and when these are analyzed by chromatography the order in which they appear in the chromatogram is betanin, isobetanin and neobetanin (Slatnar *et al*., 2015). The analyses for changes in betacyanins are summarized in Table 5, where the areas of the four peaks found in the chromatograms are reported.

Table 5. Individual betacyanins of encapsulated of beetroot stem powder using different levels of *A. vera* as carrier and different spray drying temperatures.
Tabla 5. Betacianinas individuales de polvo encapsulado de tallo de betabel utilizando diferentes contenidos de *A. vera* como acarreador a diferentes temperaturas de secado.

Temperature (ºC)	Mixture (A-B)	Peak 1 (mAU)	Peak 2 (mAU)	Peak 3 (mAU)	Peak 4 (mAU)
150	25:75	3.50 ± 0.08[a]	10.93 ± 0.62[a]	1.44 ± 0.10[a]	0.54 ± 0.02[a]
180	25:75	2.53 ± 0.23[b]	6.10 ± 0.11[b]	1.03 ± 0.05[b]	0.35 ± 0.01[b]
150	75:25	nd	nd	nd	nd
180	75:25	nd	nd	nd	nd

Area mean ± standard deviation. A (%) = *A. vera*; B (%) = beetroot steam juice; nd = not detected. Means with different letter by peak indicate significative ($p<0.05$) difference, by Tukey's test.

In the analysis of betacyanins in beetroot juice, betanin has been reported as the first peak in chromatograms, being the peak with more area. However, when beetroot juice is treated thermally, the first peak has a smaller area than betanin, being a product of betanin degradation, and then betanin appears as the second peak (Mikołajczyk-Bator & Pawlak, 2016). Then in Table 5, peak 2 could be betanin and the peak 1 is a product of its degradation; both peaks (44.19 and 27.7 %, respectively) decreased significantly ($p<0.05$) due to an increase in drying temperature for encapsulated powder obtained with 25 % of *A. vera*. Peaks 3 and 4, may be related to some modified structure of betanin, isobetanin or neobetanin. They are smaller areas than betanin (peak 2) and peak 1, but these were also affected by the increase in drying temperature with reductions of 26.1 and 33.9 %, respectively. Tabio-García *et al*. (2022) reported that betacyanin losses occur in Amaranthus at 150 ºC during spray drying.

On the other hand, no peaks were found in the encapsulated powders obtained with 75 % of *A. vera* in the mixture. This is due to the fact that the content of beetroot stem juice is low and consequently the content of betacyanins is also low, and in addition the drying temperature causes decreases or changes in these pigments. While that, betaxanthins (yellow-orange pigments) are the minority group, composed of vulgaxanthin I and II, as well as miraxanthin V. However, due to their low content, no peak could be detected during the analysis, for which a wavelength of 481 nm was used. It has been reported that betaxanthins are more affected by the effect of temperature than betacyanins (Ruiz-Gutiérrez *et al*., 2014; Mikołajczyk-Bator & Pawlak, 2016).

Regarding the antioxidant activity of the encapsulated powders, the results are shown in Table 6, this property had a similar behavior to betalains. The differences were related to the *A. vera* used in the mixture. The highest antioxidant activity values were around 1.3 mmol TE/100 g for encapsulated powders with 25 % of *A. vera*, for both drying temperatures, being statistically different from powders with 75 % *A. vera* dried at 150 ºC with values of 0.18 mmol TE/100 g. The values of antioxidant activity of encapsulated powders are lower than the antioxidant activity of beetroot stem juice and *A. vera* gel (Table 1), due to degradation or transformation of compounds as betalains and others as polyphenols (Tabio-García *et al*., 2022).

Table 6. Antioxidant activity of encapsulated beetroot stem powder with different content of A. *vera* as carrier and different spray drying temperature.
Tabla 6. Actividad antioxidante de polvo encapsulado de tallo de betabel utilizando diferentes contenidos de *A. vera* como acarreador a diferentes temperaturas de secado.

Temperature (ºC)	Mixture (A-B)	Antioxidant activity (mmol TE/100 g)
150	25:75	1.33 ± 0.13[a]
180	25:75	1.36 ± 0.03[a]
150	75:25	0.18 ± 0.05[b]
180	75:25	nd

Area mean ± standard deviation. A (%) = *A. vera*; B (%) = beetroot steam juice; nd = not detected. Means with different letter by peak indicate significative ($p<0.05$) difference, by Tukey's test.

4. Conclusion

The use of *A. vera* gel as a carrier in spray drying led to the obtaining of encapsulated beetroot stem juice powders. The results show that with increasing *A. vera* decreased H, pH, BD, T_g, WSI, BC, BX and the tendency to red, while luminosity and tendency to yellow increased, presenting more compressed capsules in the powders. Temperature affected the WSI, the color parameters a* and b* and the individual betacyanins, reducing the individual betacyanins and consequently the tendency to red. The encapsulated powders obtained showed characteristics that allow the stability of the powder components, such as low a_w (<0.13) and low moisture content (<10 %), pH suitable for the stability of betalains (5.3-5.88) and T_g suitable for storage (around 50 ºC). Highlighting that the powder obtained with 25 % *A. vera* and at a drying temperature of 180 ºC presented the best characteristics to be used as an ingredient in food formulation, with a water solubility index of 86.6, a color with a tendency to red-purple and with a content of ≈1.3 mg/g and ≈0.68 mg/g of betacyanins and betaxanthins, respectively, representing an alternative as a natural pigment to give or improve the color in food products.

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Interes conflict

The authors declare no conflict of interest.

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