Vol. 23, No. 2(2024) Alim24247
Revista Mexicana de Ingeniería Química

Microencapsulation of betalains obtained from garambullo fruit (*Myrtillocactus geometrizans*) by spray drying

Microencapsulación de betalainas obtenidas del fruto de garambullo (*Myrtillocactus geometrizans*) por secado por aspersión

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Received: January 19, 2024; Accepted: May 9, 2024

Abstract
Garambullo (*Myrtillocactus geometrizans*) is a cactus whose fruits are rich in phytochemical compounds, including betalains, and is distributed in central Mexico. It has beneficial effects on the organism due to its bioactive compound content, which has shown hypoglycemic and hypocholesterolemic effects. However, because of its short shelf life, it has not been widely commercialized and exploited, so an alternative for developing a viable technique to protect its bioactive compounds was proposed. The study's objective was to microencapsulate the bioactive pigments of garambullo by spray drying, using starch and maltodextrin as encapsulating agents to obtain microcapsules that provide antioxidant and natural pigmentation properties for industrial use. The formation of the microcapsules was carried out by preparing an emulsion, with 36.80% garambullo juice, 3.17% *Aloe vera* mucilage, 23.70% maltodextrin, 31.60% starch and 4.73% SiO_2 as encapsulating agents. Three different spray dryer temperatures (140°C, 160°C, and 180°C) were used to evaluate the best temperature. The betalain content of the encapsulates was measured at 538 nm, and the best inlet temperature was 160°C at 0. 0062 mg/100 g of sample.
Keywords: Garambullo, betalain, microcapsules, emulsion, temperature.

Resumen
El garambullo (*Myrtillocactus geometrizans*) es una cactácea cuyos frutos son ricos en compuestos fitoquímicos, entre ellos las betalaínas, se distribuye en el centro de México. Tiene propiedades benéficas para el organismo debido al contenido de compuestos bioactivos, los cuales han mostrado efectos hipoglucemiantes e hipocolesterolémicos. Sin embargo, es poco comercializado y explotado debido a su vida de anaquel corta, por lo que se propone la alternativa de desarrollar una técnica viable para proteger sus compuestos bioactivos. El objetivo del estudio fue microencapsular los pigmentos bioactivos del garambullo mediante secado por aspersión, utilizando almidón y maltodextrina como agentes encapsulantes para obtener microcápsulas que proporcionen propiedades antioxidantes y de pigmentación natural para uso industrial. La formación de las microcápsulas se llevó a cabo preparando una emulsión, con un 36.80% de jugo de garambullo, 3.17% de mucílago de *Aloe vera*, 23.70% de maltodextrina, 31.60% de almidón y 4.73% de SiO_2 como agentes encapsulantes. Se utilizaron tres temperaturas diferentes del secador por aspersión (140°C, 160°C y 180°C) para evaluar la mejor temperatura. El contenido de betalaína en encapsulados se midió a 538 nm y se observó que la mejor condición de temperatura de entrada era 160°C al tener 0.0062 mg/100 g de muestra.
Palabras clave: Garambullo, betalaína, microcápsulas, emulsión, temperatura.

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https://doi.org/10.24275/rmiq/Alim24247
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

Garambullo, whose scientific name is *Myrtillocactus geometrizans*, is a cactus (Nava-Martinez, 2023). It is widely distributed in central Mexico and, according to studies, it has been shown that garambullo can be considered a functional food because of its content of bioactive compounds, which are hypoglycemic and hypocholesterolemic (Aldana *et al*., 2004). Among these phytochemical compounds are betalains, which have important properties such as pigmentation (Otálara *et al*., 2015). Rahimi *et al*., (2019) defines betalains as natural colorants, derived from indole, consisting of a central indole derivative, composed of a central nitrogenous structure, called betalamic acid. Betalains have antioxidant activity, which is due to the phenolic groups within betalains (Flores-Mancha, 2020). In addition, it is known that natural pigments have antioxidant properties and that their presence in the diet may reduce the risk of cardiovascular disease, cancer and other diseases. associated with aging (Villaño *et al*., 2016). Unfortunately, these compounds are unstable in the presence of light, temperature, pH, enzymatic activity, and in the presence or absence of oxygen and metals, hence a process is required for the conservation of their properties (Mancha *et al*., 2019). One of the optimal processes is microencapsulation by spray drying. Microencapsulation is considered as miniature packaging; they are sealed and can release their contents at controlled rates under specific conditions (Guadarrama *et al*., 2014). While drying consists of evaporating the moisture quickly and maintaining a low temperature in the particles, the formation of microcapsules consists of the homogenization of the core materials and the encapsulating materials, creating an emulsion that is then atomized in the drying chamber (Esquivel *et al*., 2015). Spray drying has advantages such as low cost compared to other methodologies and is fast, therefore, is spray drying widely used in the food and pharmaceutical industry (Cardona and Fernandez 2020). There are several microencapsulant materials for this process, including the following mentioned materials, which were used for this work. According to the literature Molina *et al*. (2022) maltodextrins (MDs) are nonsweetening nutritive polysaccharides consisting of α-(1-4) D-glucose molecules (Özkan and Bilek, 2014) that are also used in the encapsulation process because they possess several properties such as low viscosity at high solids content, good solubility, film-forming ability and low cost (Bratovcic and Suljagic, 2019). *Aloe vera* is a cactus-like plant that contains about 98. 5% water and is rich in mucilage (Lopera *et al*., 2009) Other compounds such as carboxypeptidase, magnesium, zinc, calcium, glucose, cholesterol, salicylic acid, prostaglandin precursors (gamma-linolenic acid [GLA]), vitamins A, C and E, lignin, saponins, plant sterols and amino acids are present, while anthraquinone glycosides are present in the latex coating of the leaf: Such as aloemodin, barbaloin (Vega *et al*., 2015), aloin and emodin, which act as analgesics, antibacterial and antiviral agents (Sharrif-Moghaddasi and Res, 2008). That is why we want to take advantage of its properties by incorporating it into the encapsulating matrix. Starch is the main plant carbohydrate; it is a biopolymer and is formed by two main polysaccharides: amylose and amylopectin (Surjushe *et al*., 2008). Amylose is a linear polymer, formed by glucose linkages joined by α (1-4) bonds (Archundia-Sánchez, 2022). For this work, corn starch was used. In addition, the use of tween 20 has been reported to increase the stability of the emulsion droplets formed during the homogenization process, resulting in higher yields (Nazar *et al*., 2010). In the present work, the microencapsulation process of garambullo juice, which has a high betalain content, was optimized by the spray drying method, and the powder was subsequently characterized by physicochemical tests. The preparation of emulsions was carried out.

2 Materials and methods

2.1 Biological material

The garambullos were acquired from the community of "El Garabatillo" with geographic coordinates of longitude 100°44'56.155"north, latitude 21°18'55.444" west and is located at 2020 meters above sea level, Dolores Hidalgo in Guanajuato Mexico, during June 2022. *Aloe vera* was acquired from the community of ¨Teneria del Santuario with geographic coordinates 20.5978492, -100.7966796 in Celaya, Guanajuato.

2.2 Quantification of Betalains in juice

For the quantification of betalains in garambullo juice (*Myrtillocactus geometrizans*) the technique was performed in triplicate by the method described by Guevara *et al*. (2022) adapted from Viloria and Corbelli (2001). Ten grams of sample were weighed and grounded in an 80% v/v ethanol-water solution and the absorbances were read in an ultraviolet-visible spectrophotometer. For betacyanin at 538 nm and betaxanthins at 476 nm.

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Table 1 Formulations proposed for the experimental design.

Emulsion preparation		F 1 (%)		F 2 (%)		F 3 (%)	
AC	Juice	11.03		11.03		11.03	
		% M	% SS	% M	% SS	% M	% SS
M	MAV	5	0.95	5	0.95	5	0.95
	MD	40	5.69	37.5	7.11	35	6.64
	SiO_2	5	0.95	7.5	1.42	10	1.9
	Starch	50	9.48	50	9.48	50	9.48

M= Microencapsulate, AC=active core, F1=formulation 1, F2=formulation 2, F3=formulation 3, MD=maltodextrin, MAV=mucilage of *Aloe vera*, SS=soluble solids.

Ten grams of sample were weighed and grounded in an 80% v/v ethanol-water solution and the absorbances were read in an ultraviolet-visible spectrophotometer. For betacyanin at 538 nm and betaxanthins at 476 nm.

2.3 *Extraction and preparation of Aloe vera mucilage*

The extraction of *Aloe vera* mucilage was carried out according to Flores (2006). The stalk was trimmed manually taking care not to break the pericyclic cells that contain the acybar, and 0.15% w/w of polyoxyethylene sorbitan monooleate (Tween 80) was added to the total weight, homogenized in a blender for approximately 30 seconds according to the weight and the foam was removed, then refrigerated for at least 20 min at 4°C and centrifuged at 4000 rpm for 40 min at 4°C.

2.4 *Preparation of emulsions*

The soluble solids content in the juice was measured to adapt the percentage of total solids in the emulsion, with a limit of 30% w/v which is acceptable for spray drying. Emulsions were made by weighing the material and first adding the betalainic juice to a beaker. The *Aloe vera* mucilage was then stirred (magnetic stirrer) and the encapsulating material was gradually added. According to the corresponding formulation shown in Table 1, in which the maltedextrin and SiO_2 content were modified, the former by giving low viscosity at high solids content, good solubility and the benefit of low cost while SiO_2 was modified due to its moisture retention characteristics and generating greater stability to the microcapsules; thus providing good stability and higher or lower performance of the microcapsules, the encapsulating material was dissolved in a ratio of 12.5 mL of distilled water per 14 g of material, at room temperature and in the dark, avoiding the formation of lumps, the mixture was stirred for 30 to 40 minutes.

2.5 *Determination of pH and °Brix*

The pH was determined with a portable potentiometer (OAKTON, model PC-450; USA), and the °Brix with a manual digital refractometer (ATAGO, model PAL-1, USA).

2.6 *Microencapsulation*

Once the emulsions had been prepared, they were spray dried, at a feed temperature of 22-24°C, inlet air temperature of 140°C, 160°C and 180°C, an inlet airflow of 100 m^3/h, an air compression pressure of 40%, and an air feed flow of 480 m/h. A spray dryer Model B 290 Büchi^{TM} equipped with a vacuum pump will be used for the preparation of the microcapsules.

2.7 *Color determination*

Color determination was carried out using a Chroma Meter CR-400 (KONICA MINOLTA), and the colorimetric coordinates were evaluated according to the scale proposed by Cie: L a b chromaticity and hue degrees.

2.8 *Quantification of betalains in microcapsules*

The quantification of betalains in the microcapsules was performed according to previous methods Castellanos-Santiago (2008) with slight modifications suggested by Robles Aguilar (2016). Ten milligrams of each microcapsule were weighed, placed in a flask to be dispersed in 40 mL of distilled water under vortex agitation for 20 min, and subsequently centrifuged at 490 rpm for 5 min. The absorbance of betacyanin at 538 nm and that of betaxanthins at 472 nm, were measured in triplicate.

2.9 *Water activity of the microcapsules*

The water activity of the different formulations of microcapsules was determined with the Aqualab instrument, (3TE, Pullman, Washington), in which the microcapsules were added to the sample holder and

read at room temperature (22°C - 25ºC). The readings were taken in triplicate.

2.10 Condensed tannins

Condensed tannins were expressed as (+) catechin equivalents in mg/100 g of sample. According to the vanillin assay Desphande and Cheryn (1985), 200 mg of sample was weighed, 10 mL of methanol was added, and allowed to stand in the dark for 24 h; subsequently, the mixture was shaken for 20 min and centrifuged at 5200 rpm for 10 min. Then, an aliquot of 1 mL was taken and 5 mL of freshly prepared vanillin reagent (vanillin 1% w/v in methanol and HCl 8% v/v in methanol in a 1:1 ratio) was added. A correction blank was prepared with 1 mL of solution and 5 mL of 4% v/v HCl in methanol. This reaction was carried out at 30°C for 20 min, after which the absorbance was measured with a 64 UV/Vis, spectrophotometer (JENWAY) at 500 nm. The concentration of condensed tannins was calculated based on a standard curve of (+) catechin.

2.11 Phenolic content and antioxidant activity

2.11.1 Samples extraction

50 mg of reduced sample were weighed and added 5 mL of absolute methanol or 80% v/v methanol, vortexed for 20 min, sonicated for 4 min, centrifuged for 10 min at 5000 rpm at 4°C and filtered, recovering the filtrate and adding 5 mL of absolute methanol to the sediment. The sediment was subjected to extraction two more times. The filtrate was placed in a new phase with the previous one.

2.11.2 Phenolic content

The phenolic content was determined according to the method reported by George *et al*. (2005), with some modifications. In a microplate, 25 μL of sample, 25 μL of Folin-Ciocalteau reagent and 25 μL of sodium carbonate were added. The mixture was incubated for 30 min at 40°C, 200 μL of distilled water was added and the absorbance was read at 750 nm. The calibration curve was generated with a solution of gallic acid (GA). The results are expressed in mg gallic acid equivalents per gram of dry weight of the sample (mg AGE/g sample).

2.11.3 3ABTS antioxidant activity

The evaluation by the ABTS method was carried out according to the procedure proposed by Gonzales and Guerrero (2008). The reading was performed in a 96-well plate, in which 20 μL of Trolox + 230 μL of the working solution was added for the sample measurement. The mixture was allowed to stand for 6 min after which the absorbance was read at 734 nm. The samples were read at 4,10, 30, 60 and 90 min. The calibration curve was generated with a Trolox 800 μM solution. The results are expressed in mg Trolox equivalents per gram of dry weight of the sample (mg Trolox/g sample).

2.11.4 DPPH (2,2-Difenil-1-Picrilhidrazilo) determination

The evaluation by the DPPH method was carried out according to the procedure proposed by Brand-Willians *et al*. (1995). The reading was performed in a 96-well plate, in which 20 μL of extract and 280 μL of radical were added for sample measurement, 20 μL of absolute methanol and 280 μL of radical were used as blanks; after 30 min, the mixture was allowed to stand in the absence of light, and the reading was taken at 515 nm. The calibration curve was generated with a Trolox 800 μM solution. The results are expressed in mg Trolox equivalents per gram of dry weight of the sample (mg Trolox/g sample).

2.11.5 FRAP (Ferric Reducing Antioxidant Power) determination

The evaluation by the FRAP method was performed according to the procedure proposed by Benzie and Strain. (1996). In a 96-well microplate, 20 μL of extract and 280 μL of radical were added, and the mixture was allowed to stand for 30 min in the absence of light; the absorbance was read at 593 nm. The calibration curve was generated with a Trolox 800 μM solution. The results were expressed as mg Trolox equivalents per gram of dry weight of the sample (mg Trolox/g sample).

2.12 Optical microscopy analysis of microcapsules

Ten milligrams of each microcapsule were placed on a slide for visualization with a Leica DM5000B optical microscope, and then the micrographs were taken at 20x and 40x, with the aid of an internal photographic camera. The micrographs obtained were processed with the Image Pro-Plus software ver. 5.1.

2.13 Kinetics of sample duration in microcapsules

It was measured at time zero, when the samples were removed from the spray dryer, and measurements were taken every 7 days; for this purpose, 10 mg of microcapsules were weighed and placed in a flask and dispersed with 40 mL of distilled water under vortex agitation for 20 minutes, subsequently centrifuged at 490 rpm for 5 minutes and filtered on Whatman paper

of 0.4 μm to 0.4 μm Whatman paper to take the absorbance reading for betacyanins at 538 nm and for betaxanthin at 472 nm, the determinations were performed in triplicate. triplicate.

2.14 Statistical analysis

The results are expressed as the mean ± standard deviation of the experiments with three replicates. Statistical evaluation will be carried out by analysis of variance (ANOVA), making the comparison of means by Tukey's method with a significance level of $p < 0.05$, using Stargraphics XVI software.

3 Results and discussion

3.1 Garambullo pulp yield

Garambullo pulp was obtained using a pulper and an industrial centrifuge, in addition to the juice being obtained by filtration, which was of an intense red-violet color characteristic of garambullo. The yield of the pulp and the yield of the juice are shown in Table 2.

3.2 Quantification and characterization of betalains in the juice.

Table 3 shows the contents of betacyanins, betaxanthins and total betalains in the betalainic juice. This content of betacyanins is lower than that reported by Morales *et al*. (2023) where the extract reported for the fruit determined as maturity II (purple fruit) was 0.92 mg/100 g in, this is because that analysis was performed on the whole fruit and an extraction was performed with ethanol: water, while in this work the quantification of betalains in the juice was performed having values of 0. 49±0.09 mg/100 g, while Table 4 shows the pH and °Brix obtained for the sample, in which it can be said that the pH is lower than that reported by Capetillo *et al*. (2020) in which the fruit has a value of 5.5, both values are found in that reported by Lopez-Solorzano, (2021) in which the stability of betalains is values of 3.5 -7.

Table 2. Garambullo pulp yield.

Pulp (kg)	Pulp yield
36.46	87.5%
Garambullo pulp (g)	Garambullo juice (mL)
100	25

Table 3. Betalain content in garambullo juice.

Juice sample	Mean
Betacyanins (mg/100 g)	0.35 ± 0.06^{b}
Betaxanthins (mg/100 g)	0.15 ± 0.03^{c}
Total, betalains (mg/100 g)	0.49 ± 0.09^{a}

Table 4. Characterization of garambullo juice.

Measurement	Mean
pH	4.13±0.02
°Brix	18.8±0.10

Table 5. Yield of *Aloe vera* mucilage.

Aloe vera stalk (g)	Extract (g)	Yield (%)
1000	536.25	53.63±2.91

Table 6. Physicochemical characterization of emulsions.

F	T (°C)	pH	° Brix
1	140	4.17 ± 0.25^{ab}	24.20 ± 0.72^{abc}
	160	3.89 ± 0.11^{d}	25.77 ± 1.80^{a}
	180	4.09 ± 0.14^{abcd}	24.70 ± 0.87^{ab}
2	140	4.11 ± 0.03^{abcd}	22.53 ± 0.55^{cd}
	160	4.02 ± 0.19^{bcd}	23.13 ± 0.59^{bcd}
	180	3.94 ± 0.11^{cd}	24.47 ± 1.80^{ab}
3	140	4.23 ± 0.06^{ab}	21.50 ± 0.79^{d}
	160	4.26 ± 0.06^{a}	21.63 ± 1.29^{d}
	180	4.16 ± 0.07^{abc}	24.33 ± 0.77^{abc}

F=Formulation.

3.3 Aloe vera mucilage yield

A yellowish-colored *Aloe vera* mucilage was obtained, as shown in Table 5.

The 536.25 g of *Aloe vera* mucilage extract per 1000 g of stalk was good compared to the result obtained Sanchez and Santa (2009), where the yield of *Aloe vera* mucilage was 62% on a wet basis, representing a difference of approximately 10.4%, possibly due to slight modifications at the time of recovery. This could also be due to the type of *Aloe vera*, the location of cultivation and the amount of irrigation water provided to the plant.

3.4 Characterization of the emulsions of betalainic juice, Aloe vera mucilage, maltodextrin, starch and silicon dioxide

The emulsions were tested for pH and degree Brix, the results of which are shown in Table 6.

According to Capetillo *et al*. (2020) betalains are stable in the pH range of 3.5-7, the range in which most foods are found; outside this range, color

Figure 1. Spray drying of emulsions.

Table 7. Yields of juice microcapsules at different temperatures.

F	T (°C)	Yield
1	140	33.78±5.99^{c}
	160	42.91±6.89^{b}
	180	60.44±9.77^{a}
2	140	38.43±2.94^{bc}
	160	39.86±1.97^{bc}
	180	39.03±0.95^{bc}
3	140	38.67±1.53^{bc}
	160	40.58±2.55^{bc}
	180	40.72±3.26^{bc}

F=Formulation.

decreases. These compounds are found at pH 5.5-5.8, while in anaerobic conditions at pH 4.0-5.0, they are stable; moreover, when the °Brix is lower than 30, which is the acceptable limit in the spray dryer, they are suitable for processing according to Manzanarez *et al*. (2020) , increasing the maltodextrin content increases the soluble solids content, which can be observed in formulation 1, which has a higher °Brix content.

3.5 Microencapsulation and yield of microcapsules

Spray drying of the emulsions described above was carried out at 3 drying temperatures: 140 °C, 160 °C and 180 °C.

The encapsulation yield or efficiency was determined as the percentage ratio of the total mass of the product (microcapsules) recovered after spray drying to the mass of emulsion introduced to the system as shown in Table 7.

The highest yield was obtained with formulation 1 at 180 °C, whose yield was 60.4385. Arrazola *et al* (2014) mention that the process yield increased with increasing inlet air temperature. This is because higher inlet temperatures provide higher thermal efficiency and improve mass transfer processes, which leads to higher process yields. furthermore, following Buitrón and Ruales (2023) maltodextrin is used as a material carrier to remove stickiness and increase the glass transition temperature of the mixture.

3.6 Color analysis in microcapsules

Due to the microencapsulation in which the shell is formed by different encapsulating agents that are mostly white, it would be expected that the microcapsule would be of this color and the interior would be of the characteristic color of the garambullo juice. However, the obtained microcapsules were pink due to adsorption and desorption phenomena during the drying process. Table 8 summarizes the colorimetry data of the microcapsules formed by the garambullo juice where the color parameter given by L*, represents luminosity, which ranges from opaque to luminous and has values from 0 to 100. A positive value of a* is indicative of a red color and a negative value represents a green color. The positive parameter b* is a measure of the yellow color and a negative value of the blue color. The parameter c* indicates chromaticity, considered as saturation, intensity, or purity and h° indicates hue, tint, or color, it is characterized by the wavelength of the radiation and makes a color different from another.

Table 8. Color parameters of the microcapsules.

T (°C)	L*	a*	b*	C*	h*
140	84.62±1.2^{ab}	17.66±0.84^{b}	3.24±0.31^{d}	17.96±0.88^{b}	349.61±0.52^{e}
160	68.91±2.46^{b}	23.43±7.70^{a}	3.36±0.11^{d}	23.68±7.64^{a}	352.39±2.13^{de}
180	85.97±3.4^{ab}	13.51±1.39^{b}	1.95±0.22^{c}	13.65±1.41^{b}	351.77±0.57^{d}
140	83.59±2.85^{ab}	15.91±1.07^{b}	-0.94± 0. 18^{a}	15.94±1.08^{b}	356.64±0.39^{a}
160	87.07±2.36^{ab}	13.7±2.12^{b}	1.19±0.07^{b}	13.83±2.11^{b}	355.02±0.52^{a}
180	78.8±5.06^{ab}	15.58±0.94^{b}	-0. 87±0.34^{a}	15.61±0.95^{b}	356.84±1.14^{a}
140	82.98±1.72^{ab}	13.95±0.63^{b}	1.42±0.19^{b}	14.02±0.64^{b}	354.21±0.55^{bc}
160	69.47±2.53^{ab}	13.30±2.18^{b}	-0. 89±0.39^{a}	13.34±2.15^{b}	355.93±2.16^{ab}
180	76.33±6.68^{ab}	17.30±0.99^{b}	1.93±0.73^{c}	16.30±0.03^{b}	353.11±0.26^{cd}

It can be observed that the microcapsules with higher luminosity are those of formulation 2 at 160°C, this is because according to Santiago and Naturales, (2018) the presence of maltodextrin at higher concentrations tends to increase the value of L* however they have a higher value compared to formulation 1, this due to having SiO_2, since according to Carbajal *et al.* (2019) the SiO_2 increases the luminosity.

3.7 Quantification of betalains microcapsules

Betacyanins do not show a statistically significant variation regardless of the temperature used, however, in formulations 1, 2 and 3 at 180 °C there is a significant difference. The highest betacyanin content was 0.0062 mg/100 g of sample, which corresponds to the microcapsules elaborated using formulation 2 at a temperature of 160 °C. The microcapsules with the highest content of betaxanthins 0.0063 mg/100 g of sample, also with formulation 2 at 160°C, as shown in Figure 3 resulting in a higher content of total betalains in this formulation and at this temperature, which can be observed in Figure 4. These values are inferred to those reported by Castro *et al.* (2015) in which they encapsulated betalains from opuntia stricta, using gelatin-maltodextrin, giving values of 11-35 mg/100 g of sample, as well as in the case of Flores *et al.* (2020) in which they encapsulated Beta vulgaris extract using Maltodextrin and Inulin with values of 130 mg/100 g of sample, this may be due to the source of the betalains, which has a lower content than these other matrices, in addition to the encapsulation material and the conditions used for the method.

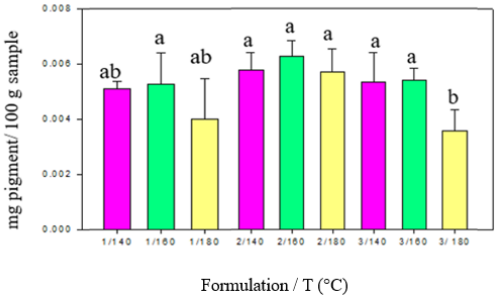


Figure 2. Betacyanin content in the microcapsules.

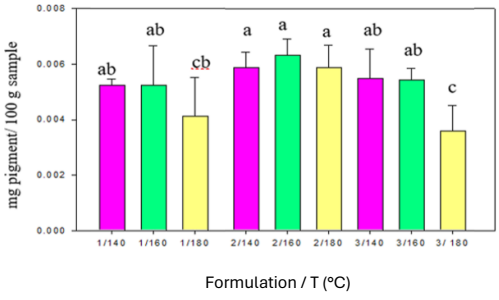


Figure 3. Betaxanthin content in the microcapsules.

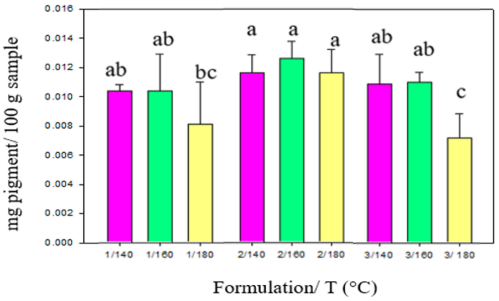


Figure 4. Total betalain content in the microcapsules.

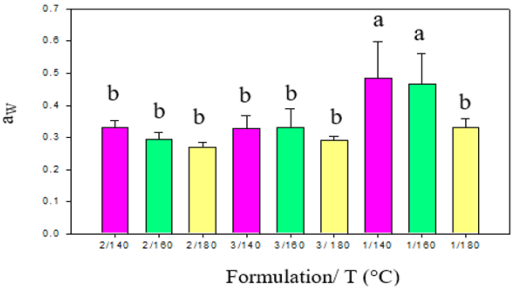


Figure 5. Water activity in microcapsules.

3.8 Water activity of microcapsules

Most of the tests presented a water activity (aw) value between 0.27 and 0.4673 as shown in Figure 5, which is a range that is below the minimum water activity required for the growth and proliferation of pathogenic microbial (a_w < 0.46) Gómez *et al.* (2021) Furthermore, water activity (aw) also influences the stability of betalains, which are more stable in foods or model systems with low moisture content and low aw, because water is less available for chemical reactions to occur. a_w values below 0.63 improve the stability of betalains Morales *et al.* (2023). Gonzalez *et al.* (2022) mention that at a temperature of 160 °C an a_w of 0.21 is obtained when MD10 is used, values that are very similar to those obtained in the work. In the drying process, the particles after being pulverized are separated from the water molecules; however, the forces generated on the surface of the microcapsules must be considered, since the presence of polysaccharides generates interactions, such as van der Waals or polymer-induced forces, which in turn generate additional forces that can be repulsive or attractive to molecules such as water. Therefore, the solids obtained will have water molecules bound and/or trapped on their surface after the drying process.

3.9 Condensed tannins

Condensed tannins (CT) or proanthocyanidins are secondary compounds of high molecular weight that protect plants from pathogens, insects, and herbivores. These polymers are formed by 4 subunits of monomers

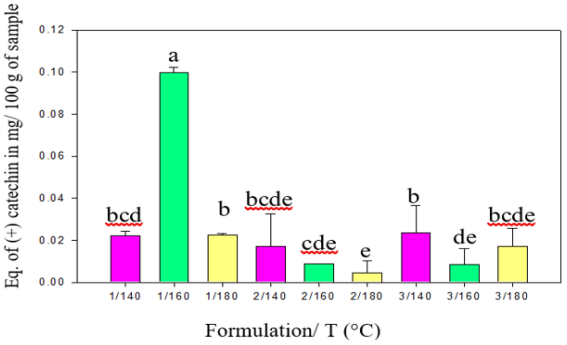


Figure 6. Tannin content of condensed tannins in microcapsules.

(flavan-3-ol). In turn, these polymers are divided into proanthocyanidins, when the flavan-3-ol structure is catechin and epicatechin, or prodelphinidins when the flavan-3-ol structure is gallocatechin or epigallocatechin Álvarez *et al*. (2020). The results of the condensed tannin content in the microcapsules were expressed as catechin equivalents (mg EC/100 g) as shown in Figure 6 and it can be observed that the formulation had a considerable influence on its content, for formulation 1 the highest values were observed at a temperature of 160°C, while for formulation 2 when increasing the temperature a loss of these was observed and finally in formulation 3 the lowest value was observed when working at 160°C.

These results vary concerning those presented by Arrefonfo *et al*. (2020) in which he analyzed the content of condensed tannins in different stages of ripening of garambullo, for the mature stage, which was the same stage in which it was handled in this work reported) 5.52 ± 0.35 mg EC g-1 sample, the variation of the results may be due to the origin of the fruit in addition to the storage conditions.

With the results obtained based on the content of betalains in the microcapsules, it can be said that formulation 2 in its three temperatures is the best, statistically, the three temperatures of this formulation did not show a significant difference, however, there is a higher content at the temperature of 160°C and has a higher yield since it did not show a significant difference in terms of betalain content, therefore, the analysis was continued only with this formulation to determine the best temperature.

3.10 Antioxidant activity

Antioxidant activity was measured by three methods, DPPH, FRAP and ABTS, with the FRAP methodology showing the highest activity, followed by ABTS and finally DPPH. For these and the following analyses, only formulation 2 was analyzed at a temperature of 160°C since it is the formulation with the highest betalains content. As shown in Figure 7, in all three methods, the highest values were

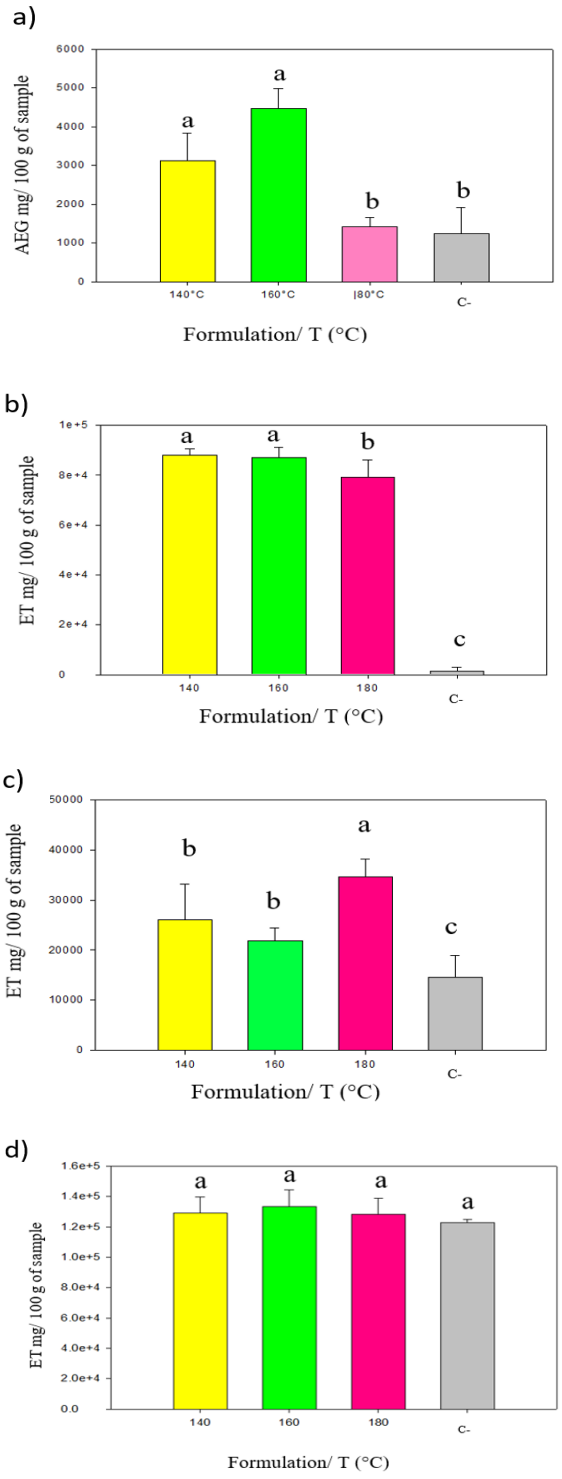


Figure 7 a) Total phenols b) Antioxidant activity determined by the DPPH method, c) Antioxidant activity determined by the ABTS method, d) Antioxidant activity determined by the FRAP method. C-: negative control.

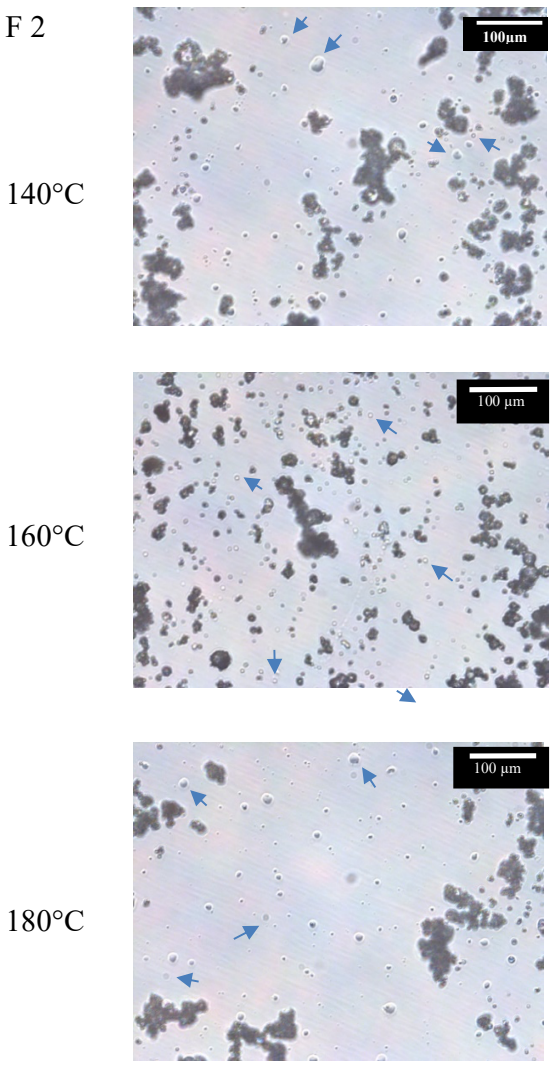
obtained at a temperature of 160°C. It should be noted that garambullo was in a mature state when used. According to Lopez *et al*. (2019) garambullo at different stages of maturity, antioxidant activity was analyzed was preserved due to the presence of

betalains since these pigments increase during fruit ripening.

Compared with the results of Frisancho *et al*., (2023), in which the antioxidant activity was 41.1%, the highest value was obtained with the GA, extract because the analysis was performed on fruit in addition to the microencapsulation process and the temperatures.

3.11 Optical microscopy analysis

As part of the analysis of the microcapsules containing betalains from garambullo, a physical examination was performed by optical microscopy to determine their physical characteristics. Each of them was seen at a magnification of 40x in Figure 8, the microcapsules can be observed at different temperatures, showing that some of the microcapsules are empty in the nucleus, showing this tendency in those of 140 and 180°C, while in those of 160, they are shown with greater content in their nucleus.



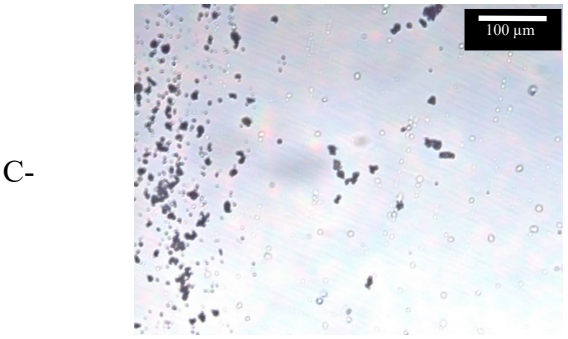


Figure 8. Effect of temperature on microcapsule formation, 40x optical microscope evaluation.F=Formulation. C-=Negative control.

3.12 Kinetics of betalains release from microcapsules

Figure 9 shows a decreasing trend in the release profile of betalains from the microcapsules at 140°C and 180°C. However, at 160°C, betalains were released at an increasing rate starting at day 10, at which time the maximum release of betalains was greater than 0.016 mg /100 g of sample. This same trend was also observed for betacyanins (Figure 9) and betaxanthins (Figure 10) Albarran-Corona (2023) mentions that the release of the active compound in the microcapsule is influenced by the concentration gradient and the attractive forces between chains, such as hydrogen bridges, Van der Waals forces, degree of crosslinking and crystallinity. In addition, the release is controlled by the solubility and permeability of the core material in the protective material, while Aldana *et al*. (2004) mentions that the stability of the capsule depends on the transition temperature of the capsule. The stability of the capsule depends on its transition temperature being higher than the storage temperature. storage temperature, thus the core material is released by diffusion at a rate that increases with increasing temperature. Figures 9, 10 and 11 show that on days 15 and 20 there is a greater release on those days, which could be attributed to this factor.

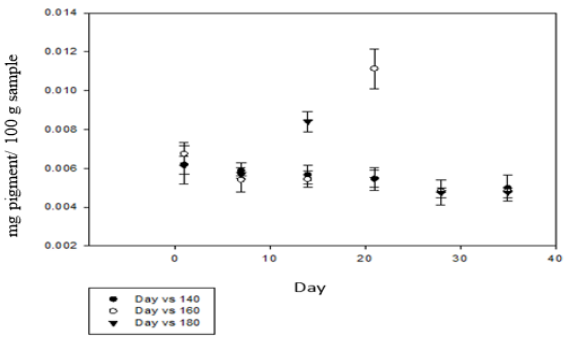


Figure 9. Kinetics of betacyanin release in microcapsules.

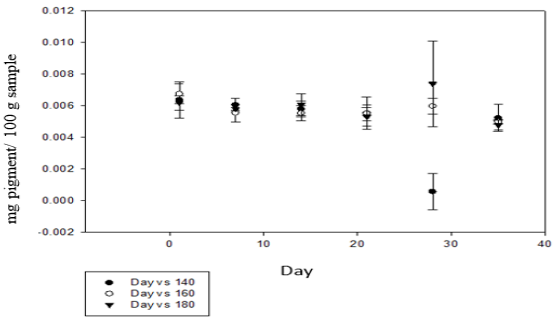


Figure 10. Kinetics of betaxanthin release in microcapsules.

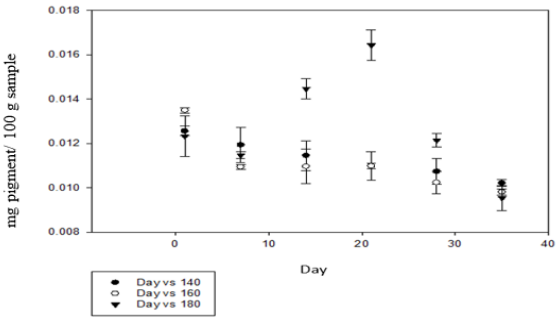


Figure 11. Kinetics of betalain release in microcapsules.

Based on the results obtained, formulation 2 is considered the best formulation for preserving betalains, at a temperature of 160°C.

Conclusion

The formulation 2 of microcapsules based on maltodextrin, starch, Tween 20, SiO_2 and *Aloe vera* mucilage, at a temperature of 160°C, allowed a better preservation of betalains given the concentration of the encapsulating matrix. Similarly, there was a high content of phenolic compounds with a value of 44%. Phenolic compounds, with a value of 4475 mg EAG/100 g sample.

Acknowledgements

The author thanks Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT) for the scholarship no. 808771 granted to Daniela Gómez Espinoza allowed the fulfillment of this work.

References

Albarrán-Corona A.L. (2023). Encapsulación en la industria de alimentos (Tesis de pregrado). Universidad autónoma del Estado de México. Toluca, Estado de México.

Aldana A. S., Sandoval, E. R. and Aponte, A. A. (2004). Encapsulación de aditivos para la industria de alimentos. *Ingeniería & Competitividad 5*(2), 73-83. https://doi.org/10.25100/iyc.v5i2.2298

Álvarez-Rodríguez, J., Serrano-Pérez, B., Villalba, D., Joy, M., Bertolín, J. R., & Molina, E. (2020). ¿Afectan los taninos condensados de la dieta a los resultados productivos, la composición de ácidos grasos y el color de la carne de cordero? (No. ART-2020-118645).

Archundia Sánchez, A. (2022). Evaluación del almidón de maíz doblemente modificado como agente encapsulante del extracto de *Hibiscus sabdariffa*. Tesis de maestría en ciencia y tecnología de alimentos. Universidad Autonoma del Estado de Hidalgo. Tulancingo de Bravo, Hidalgo, México.

Arrazola, G., Herazo, I., & Alvis, A. (2014). Microencapsulación de antocianinas de berenjena (*Solanum melongena* L.) mediante secado por aspersión y evaluación de la estabilidad de su color y capacidad antioxidante. *Información Tecnológica 25*(3), 31-42.

Arredondo, J. M. F., López, A. P. and Guerra, R. D., (2020) Caracterización física y bioquímica de frutos de garambullo (*Myrtillocactus geometrizans*). *Química, Etnobotánica, Economía y Finanzas, 8*.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry 239*(1), 70-76

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology 28*(1), 25-30.

Bratovcic, A. and Suljagic, J. (2019). Micro-and nano-encapsulation in food industry. *Croatian Journal of Food Science and Technology 11*(1), 113 121. https://doi.org/10.17508/CJFST.2019.11.1.17

Buitrón F. and Ruales J. (2023). Development of a soluble antioxidant beverage of tree tomato (*Solanum betacea*) for sportsmen. *London Journal of Research in Science: Natural and Formal 23*(18).

Capetillo Rubio A., Lira Vargas A., Pascual Bustamante S. and Trejo Márquez M.A.

(2020) Elaboración de bebida carbonatada de garambullo (*Myrtillocactus geometrizans*) aplicando ozono y sonicación como métodos alternativos a la pasteurización. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos 5* (2020) 426-431

Carbajal-Vázquez, V. H., Trejo-Téllez, L. I., Gómez-Merino, F. C., Alcántar-González, G., Sánchez-García, P., Ramírez-Olvera, S. M., & de la Luz, M. (2019). Dióxido de silicio mejora el color y la concentración de licopeno en tomates sometidos a estrés salino.Desarrollo científico en México.

Cardona, C. E. O., and Fernández, X. A. (2020). Quitosano: una alternativa sustentable para el empaque de alimentos. *Revista Digital Universitaria 21*(5).

Castellanos-Santiago E, E.M Yahia (2008) Identification and quantification of betalains from the fruits of 10 Mexican prickly pear cultivars by high-performance liquid chromatography and electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry 56*, 5758-5764

Castro-Muñoz, R.; Barragán-Huerta, B.; Yáñez-Fernández, J. (2015). Use of gelatin-maltodextrin composite as an encapsulation support for clarified juice from purple cactus pear (*Opuntia stricta*). *LWT-Food Science and Technology 62*, 242-248.

Desphande, S.; Cheryan, M. 1985. Evaluation of vanillin assay for tannin analysis of dry beans. *Journal of Food Science 50*(4), 905-916. doi: 10.1111/j.1365-2621.1985.tb12977.x

Esquivel-González, B. E., Martínez, L. O., and Rutiaga-Quiñones, O. M. (2015). Microencapsulación mediante secado por aspersión de compuestos bioactivos. *Revista Iberoamericana de Tecnología Postcosecha 16*(2), 180-192. id=81343176006

Flores-Mancha, M. A., Ruíz-Gutiérrez, M. G., Sánchez-Vega, R., Santellano-Estrada, E., & Chávez-Martínez, A. (2020). Characterization of betabel extract (*Beta vulgaris*) encapsulated with maltodextrin and inulin. *Molecules 25*(23), 5498.

Frisancho, J. P. P., Velarde, F. J. S., Panca, P. Z., Ramos, L. M. M., Sarmiento, P. K. D., & Pacheco, H. G. J. (2023). Microencapsulación de betacianina de *Opuntia ficus-indica* mediante liofilización y efecto en estabilidad y actividad antioxidante. *Revista Cubana de Farmacia 56*(1).

George, S.; Brat, P.; Alter, P. and Amiot, M. J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agriculture and Food Chemistry 53*, 1370-1373.

Gómez-Fernández, A. R., Faccinetto-Beltrán, P., Orozco-Sánchez, N. E., Pérez-Carrillo, E., Santacruz, A., and Jacobo-Velázquez, D. A. (2021). Physicochemical properties and sensory acceptability of sugar-free dark chocolate formulations added with probiotics. *Revista Mexicana de Ingeniería Química 20*(2), 697-709.

González, A., & Guerrero, J. (2008). Betalaínas: importancia, presencia en vegetales y sus aplicaciones en la industria alimentaria. Departamento de Ingeniería Química y Alimentos, 1-9.

González-González, M., Chong-Canto, S., Cruz-Gorostieta, Y. A., Ruiz-Palma, M. S., and Cerón-Montes, G. I. (2022). Evaluación de nano secado por aspersión a alta concentración de alimentación. *Pädi Boletín Científico de Ciencias Básicas e Ingenierías del ICBI, 10*(Especial7), 172-177.

Guadarrama-Lezama, A. Y., Cruz-Olivares, J, Martínez-Vargas, S. L., Carrillo-Navas, H., Román-Guerrero, A., and Pérez-Alonso, C. (2014). Determination of the minimum integral entropy, water sorption and glass transition temperature to establishing critical storage conditions of beetroot juice microcapsules by spray drying. *Revista Mexicana de Ingeniería Química 13*(2), 405-416.

Guevara, J. A. Z., Mori, J. R. C., Jiménez, W. J. J., and Santi, W. E. M. (2022). Actividad antioxidante de pulpa, semilla y pericarpio de mazorca del *Theobroma cacao*. *RECIAMUC 6*(3), 564-574.

Lopera, S. M., Guzmán, C., Cataño, R. C., and Gallardo, C. (2009). Desarrollo y caracterización de micropartículas de ácido fólico formadas por secado por aspersión, utilizando goma arábiga y maltodextrina como materiales de pared. *Vitae 16*(1), 55-65.

López Solórzano, E. (2021). Estabilización de betalaínas de betabel para prolongar su vida de anaquel. (Tesis de maestría). Instituto Tecnológico de Toluca. Metepec, Estado de México

López-Palestina, C. U., Aguirre-Mancilla, C. L. A., Ramírez-Pimentel, J. G. A., Raya Pérez, J. R. A., Santiago Saenz, Y. O. B., Gutiérrez-Tlahque, J. C. and Hernández Fuentes, A.

(2019). Compuestos bioactivos y actividad antioxidante en tres estados de madurez de *Myrtillocactus geometrizans* provenientes del Valle del Mezquital, Hidalgo. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos 4*.

Mancha, M. A. F., Monterrubio, A. L. R., Vega, R. S., and Martínez, A. C. (2019). Estructura y estabilidad de las betalaínas. *Interciencia 44*(6), 318-325

Manzanarez-Tenorio, L. E., Ruiz Cruz, S., Márquez-Ríos, E., Ornelas-Paz, J. de J., Del-Toro-Sánchez, C. L., Wong-Corral, F. J., Suárez-Jiménez, G. M., Dévora-Isiordia, G. E., and Holguin-Soto, R. (2020). Microencapsulación de extractos de higo (*Ficus carica*) por coacervación compleja y evaluación de su capacidad antioxidante. *Biotecnia 22*(2), 70-77. Epub 07 de agosto de 2020. https://doi.org/10.18633/biotecnia.v22i2.1247

Molina, J. O. R., Castellano, A. M. T., Ochoa, Z. E. Z., Chérrez, D. L. I., & Molina, P. E. P. (2022). Microencapsulación del extracto acuoso de cedrón (*Aloysia citrodora*) mediante secado por aspersión. *UTCiencia 9*(2), 99-112.

Morales-Domínguez, J. F., Herrera-Martínez, V., Balch, E. M. P. M., and Garcidueñas-Piña, C. (2023). Estudios de los efectos del pH, densidad estomática, ácido-3-indolacético y ácido abscísico en cactáceas cultivadas *in vitro* y *ex vitro*. *Investigación y Ciencia de la Universidad Autónoma de Aguascalientes*, (88).

Nava Martínez, L. H. (2023). Recolección de alimentos vegetales: una tradición chichimeca en Zacatecas. Cuicuilco. Revista de ciencias antropológicas, 30(87), 43-72. Epub 17 de noviembre de 2023. Recuperado de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2448-84882023000200004&lng=es&tlng=es.

Otálora, M. C., Carriazo, J. G., Iturriaga, L., Nazareno, M. A., and Osorio, C. (2015). Microencapsulation of betalains obtained from cactus fruit (*Opuntia ficus-indica*) by spray drying using cactus cladode mucilage and maltodextrin as encapsulating agents. *Food Chemistry 187*, 174-181. https://doi.org/10.1016/j.foodchem.2015.04.09

Özkan, G., & Bilek, S. E. (2014). Microencapsulation of natural food colorants. International Journal of Nutrition and Food Sciences, 3(3), 145-156.

Rahimi, P., Abedimanesh, S., Mesbah-Namin, S. A., and Ostadrahimi, A. (2019). Betalains, the nature-inspired pigments, in health and diseases. Critical reviews in food science and nutrition, 59(18), 2949-2978. https://doi.org/10.1080/10408398.2018.1479830

Robles-Aguilar M. V. (2016). Contenido de betalainas y actividad antioxidante en brácteas de bougainvillea glabra choisy. Universidad Técnica de Machala.

Sánchez A. I. and Santa C.J. (2009). Estudio de antraquinonas presentes en extractos de mucílago y hojas de *Aloe vera* de plantas cultivadas en la región cafetera. Universidad Tecnológica de Pereira. Facultad de Tecnología Química.

Santiago and Naturales, (2018). Secado de jugo de jitomate por aspersión con coadyuvantes y antioxidantes naturales para mejorar la retención de compuestos bioactivos. Repositorio Nacional Conacyt

Sharrif Moghaddasi, M., and Res, M. (2011). *Aloe vera* their chemicals composition and applications: A review. *International Journal of Biological Medical Research 2*(1), 466-71.

Surjushe, A., Vasani, R., and Saple, D. G. (2008). *Aloe vera*: a short review. *Indian Journal of dDermatology 53*(4), 163. doi: 10.4103/0019-5154.44785

Vega, A., Ampuero, N., Díaz, L., and Lemus, R. (2005). El *Aloe vera* (*Aloe barbadensis Miller*) como componente de alimentos funcionales. *Revista Chilena de Nutrición 32*(3), 208-214. http://dx.doi.org/10.4067/S0717-75182005000300005

Villaño D., Garcia-Viguera C. and Mena P. (2016). Colors: Health Effects. In: Caballero, B., Finglas, P., and Toldra, F, (eds.) *The Encyclopedia of Food and Health 2*. Pp 265-272. Oxford Academic Press

Viloria M. A., Corbelli M. D. (2001). Evaluación del contenido y estabilidad de las betalaínas en pulpa de fruta *Optunia boldinghii* Br. Et. (Dissertation) Canoabo. Universidad Nacional Experimental Simón Rodríguez 70 p.