Chapter 10 Microencapsulation of acachul (*Ardisia Compressa)* extract by spray drying using diferente polymeric materials as encapsulating agents

Capítulo 10 Microencapsulación de extractos de acachul (*Ardisia Compressa*) mediante secado por aspersión utilizando diferentes materiales poliméricos como agentes encapsulantes

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Abstract

The microencapsulation process is a technique whose purpose is to protect liquid, solid and gaseous compounds susceptible to thermal, light or oxidative deterioration, among other factors. The particular substance may be individually coated with an encapsulating material to protect it from the environment, from the reaction with other compounds or to prevent oxidation reactions from light or oxygen present in its surroundings. There is a wide variety of biopolymeric materials used as barrier materials for encapsulation. Among the materials that serve as encapsulating or entraining agents are carbohydrates, lipids, proteins and polymers, while the active or encapsulated compounds may be antimicrobials, pigments, vitamins, minerals or microorganisms. Therefore there is a need to find the best option, to encapsulate the desired active ingredients using different biopolymeric materials as barrier materials. Whence, in the present work, the effect and characteristics from acachul (*Ardisia Compressa*) pigments were determined, encapsulated them using maltodextrin, gum arabic and a combination of gum arabic- maltodextrin in a 1:1 ratio as encapsulating agents. Determining that acachul (*Ardisia Compressa*) pigments were better encapsulated when maltodextrin was utilized as the encapsulating agent.

Microencapsulation, Pigments, Encapsulation agents, Acachul

Resumen

El proceso de microencapsulación es una técnica que tiene como finalidad proteger, líquidos, sólidos y gaseosos susceptibles al deterioro térmico, lumínico, oxidativo, entre otros. La sustancia en particular puede ser cubierta de manera individual con un material encapsulante para protegerla del ambiente, de la reacción con otros compuestos o para impedir que sufran reacciones de oxidación debido a la luz o al oxígeno presentes en su entorno. Existe una gran variedad de materiales biopolímericos como materiales de barrera utilizados para la encapsulación. Entre los materiales que sirven como agentes encapsulantes o de arrastre se encuentran los carbohidratos, lípidos, proteínas y polímeros, mientras que los agentes activos o encapsulados pueden ser antimicrobianos, pigmentos, vitaminas, minerales o microorganísmos. Por lo que existe la necesidad de encontrar la mejor opción, para encapsular los ingredientes activos deseados utilizando diferentes materiales biopoliméricos como materiales de barrera. Por lo que en el presente trabajo, se determinó el efecto y características que presentan los pigmentos de acachul (*Ardisia Compressa*). Encapsulados al utilizar maltodextrina, goma arábiga y una combinación de goma arábiga-maltodextrina en proporción 1:1 como agentes encapsulantes. Determinando que los pigmentos de acachul (*Ardisia Compressa*) fueron mejor encapsulados al utilizar maltodextrina como agente encapsulante.

Microencapsulación, Pigmentos, Agentes encapsulantes, Achachul

1 Introducción

Nowadays, consumers demand nutritious and functional products that also contribute to consumer health. For such reason, food and coloring industries have been subject to making changes in reformulation of their products and searching for new alternatives that do not affect consumers health maintaining the desired characteristics of food. For this reason, natural pigments of vegetable origin (carotenoids, chlorophylls, phenolic pigments: flavonoids, anthocyanins, tannins, and betalains) have been chosen as substitutes for artificial colors. In addition to the above, Mexico has a wide range of plant sources with attractive colors that are due to the presence of compounds (anthocyanins) found in the epidermal tissues of flowers and fruits mainly, which provide red, orange, blue and purple colors. By geographical location, in north of Puebla State an endemic fruit called Acachul (*Ardisia compressa*) is produced, which is cultivated in the municipalities of Xicotepec, Tlaxco, Zihuatehutla, Tlacuilotepec, Jalpan and Pahuatlán all of them belonging to northern zone of Puebla State, Mexico (SAGARPA, 2018). This fruit is not entirety exploited, being commonly used as raw material for elaboration of artisan wines, thereby opening a field of application for obtaining natural coloring, having with it an economic and social benefit for producers of Acachul fruit expanding the market and encouraging its production to prevent this endemic crop from being lost by giving it a new added value and greater use.

Therefore, importance of this research lies fundamentally in using polymeric agents as protective coatings of interest compounds (anthocyanins) and by means of spray drying used as a micro-encapsulation technique to obtain a natural powder pigment and thuscarrying out its physicochemical characterization, determining stability conditions for its application in food systems.

2 Theoretical Fundaments

2.1 Acachul (*Ardisia compressa*)

In northern region of Puebla State, Acachul bush (*Ardisia compressa*) is cultivated, which is a plant that is still in its natural agro-ecosystem. The municipalities Xicotepec, Tlaxco, Zihuatehutla, Tlacuilotepec, Jalpan and Pahuatlán are the main producers with a production of 60 tons per year, including 30 hectares area for this activity (SAGRAPA, 2018).

Acachul (*Ardisia compressa*) belongs to *Myrsinaceae* family, it is described as a shrub 1 to 3 m high, it has round or elliptical-lanceolate leaves of 10 to 20 cm, with entire or serulated edge, with smooth textures above and below. Flowers are pinkish-white in color and produce edible globose fruit. The seed or wild fruit is similar to a bunch of grapes, which contains a single seed, its flavor is sour-sweet, similar in size to blueberry, one of its main characteristics is that it has a high content of anthocyanin compounds and can be used to obtaining a natural pigment (Vázquez., *et al*. 2019, Vázquez., *et al.* 2021).

2.2 Pigments

Color is an important factor in determining the attractiveness of most foods, color is often used as an index of freshness and good condition. Unfortunately, color can change during processing, storage, or preparation, thereby reducing food quality. So controlling, changing and stabilizing food color is one of the main goals of food scientists and technologists.

2.3 Anthocyanins

Anthocyanins, alike flavonoids and betalains, are water-soluble pigments with glycoside characteristics, they are made up of an anthocyanidin molecule, which is aglycone, to which a sugar is attached through a β-glucosidic bond. Basic chemical structure of these aglycones is the flavillium ion, which consists of two aromatic groups: a benzopyryl (A) and a phenolic ring (B); Due to its trivalent position of oxygen, flavillium normally functions as a cation (Aguilera, *et al.* 2011). Currently about 20 anthocyanins are known, the most important of which are perlargonidin, delphinidin, cyanidin, petunidin, peonidin and malvidin. It is very common that an anthocyanidin interacts with more than one carbohydrate to form different anthocyanins (Barragán, *et al*., 2018). Anthocyanins have become an interesting option for food industry, as possible substitutes for synthetic colorants. Additionally, these substances exhibit an added value due to their antioxidant and cytotoxic capacity (Cassidy, 2018). In addition to the above, the expanded interest in anthocyanic compounds has become known, knowing that they play an important role in reducing coronary heart disease, cancer, diabetes, anti-inflammatory effects and improvement of visual acuity. Likewise, they are considered potential agents in obtaining products with added value for human consumption (Garzón, 2008; Nardini & Garaguso, 2020). Consequently, demand for acquiring these compounds has increased, which leads to search for new techniques for obtaining anthocyanins on large scale, which is why the use of spray drying has been directed mainly; which has led to studies of separation, structural characterization and quantification of obtained anthocyanins (Guerra and Ortega, 2006).

2.3.1 Color and stability of anthocyanins

One of best known and most widespread functions of anthocyanins is to provide flowers with color, making them more attractive to pollinators. Considerable effort has been made to explain color variations exhibited by anthocyanins in plants; various factors such as concentration and nature of anthocyanidin, equilibrium forms of anthocyanidin, glycosylation or acylation, nature and concentration of co-pigmentation, metal complexes, intra- and intermolecular association mechanisms, and the influence of external factors such as pH, salts, among others, have been shown to have a certain impact on coloration of anthocyanins.

Role of anthocyanins and flavones to provide angiosperm flowers with a stable blue color has been recently studied, finding that anthocyanin with the greatest presence is delphinidin, and that co-pigmentation with a specific flavone was most common mechanism to transform the mauve color of delphinidin glycosides to blue hues. One of the first systematic reports on the role of pH in chemical stability of anthocyanins, found that anthocyanins slightly diluted at typical pH values of various foods, in a range of 3 to 6, resulted in being almost completely hydrated to obtain Colorless carbinol pseudobases, while the flavyl ion exists mostly at pH values below 2.0 (Nollet & Toldrá, 2013).

2.3.2 Anthocyanin degradation mechanisms

Aside from typical factors, species, agronomic and environmental factors, which affect anthocyanin content in fruits, its processing prior to consumption significantly influences total content in final product. Thermal processing is one of the most common processes, involving heating to temperatures between 50 and 150 ° C, depending on pH of the product and desired shelf life. Given their potential, technical or benefits they could bring to human health, chemical stability of anthocyanins is a focus point for many assays and analyzes.

As scrutinized, it has been observed that anthocyanins stability depends not only on processing temperature, but also on intrinsic properties of the product such as its pH, storage temperature, chemical structure and anthocyanin concentration, as well as presence of light, oxygen, enzymes, proteins, and metal ions.

2.3.3 Nutritional and health aspects related to anthocyanins

Interest in anthocyanins for use as nutritional supplements in the daily diet of humans has increased. It is postulated that regular consumption of anthocyanins and other polyphenols from fruits, vegetables, wines, jellies, and preserves, is associated with a possible reduction in risk of chronic diseases such as cancer, cardiovascular diseases, viral inhibition, and Alzheimer's (Rangel Huerta *et al.*, 2015) Anthocyanins, as well as other flavonoids, are considered important nutraceuticals due to their antioxidant effects, which give them a potential role in prevention of various diseases related to oxidative stress. There are several studies channelized to identify that anthocyanins and other flavonoids present in food have effects in the diet, analyzing their bioavailability, their metabolism, pharmacokinetics of their action, and safety of their consumption.

Current knowledge about molecular actions of anthocyanins to chemoprevent cancer is divided into: antioxidat mechanism, molecular mechanisms of anticarcinogenesis, and molecular mechanism that involves the induction of apoptosis of tumor cells (Khoo, *et al*., 2017)

2.3.4 Anthocyanins as food coloring

In addition to interest about the effects of anthocyanins on human health, there is a widespread interest in using them as food colorants. Due to the fact that these are obtained from natural sources, consumers show a certain proclivity towards this type of substances compared to synthetic colorants, which, at the same time, have limitations established by corresponding legislation. Extensive studies related to common food colorants, derived from anthocyanins, qualitative and quantitative aspects of anthocyanins used in food, and their physicochemical properties (color characteristics and stability), have been carried out along over years regarding information on the subject of application of these compounds.In cases where anthocyanins are present as part of the ingredients of the product, they undergo various transformations that lead such product acquires typical colors. Commonly, colorants that are sought to be substituted with use of anthocyanins are those that present shades between red and purple. However, biggest problem related to their use is pH control in products where they are applied, their susceptibility to thermal processing and low stability during storage. Existing legislations do not indicate specific sources from which anthocyanins, to be used as colorants, must be extracted, they simply define acceptable procedures for their extraction. Extractions carried out with acidified water, methane and ethanol are those that present a greater acceptance for this purpose. Extracts obtained by these methods are known to also contain sugars, tannins, minerals and other interfering compounds. Anthocyanin fraction, even though it can be recovered from various types of fruits and vegetables, commonly grapes, strawberry, black currant, purple cabbage and others, for economic reasons, is obtained mainly from by-products derived from the wine industry, especially the peel of grape.

A well-known commercial compound, derived from this latter matrix, distributed and used globally in Europe, Asia, and North America, is enocyanin. Extracts of black carrots, known to reach concentrations of 1,700 mg / kg of fresh product, as well as dried and deodorized extracts of purple cabbage, are also widely used in beverages, candies, ice cream, jellies and jams, in addition to light drinks (Nollet & Toldrá, 2013).

3. Methodology to be developed

3.1. Project development location

Development of this research was carried out in the city of Puebla de Zaragoza, Puebla, in the Food Laboratory facilities FIQ4 / 105 of the Chemical Engineering Department and University Center for Entailment and Technology Transfer (CUVyTT) of the Benemérita Universidad Autónoma de Puebla (BUAP).

3.2. Collection of biological material

Acachul *(Ardisia Compressa)* in a commercial maturity state, was collected in municipality of Xicotepec de Juárez, located in northwestern part of Puebla state. Fruits were stored in hermetic polyethylene bags labeled with date and place, after harvesting they were stored at -4 ° C until use for experimentation.

3.3 Characterization of fresh Acachul fruit

3.3.1 Determination of total soluble solids (TSS)

Determination of TSS was resolved in fruit stored at -4 ° C, methodology consisted of pressing a piece of fruit to extract a few drops of juice, considering the equatorial zone of fruit. Using a model PAL-1 digital refractometer at 20 ° C, prism of the refractometer was supported in a fixed place to ensure that juice is distributed evenly.

3.3.2 Color determination

Colorimetry is a non-destructive physical method widely used to determine color of a sample. To measure color (color saturation) a calibrated Hunterlab Model D25A-9000 colorimeter was used, it provides the achromatic parameters or coordinates L * wich is luminosity or clarity and represents whether a color is dark, gray or light, varying from zero to a black up to 100 for white and the chromatic coordinates a * and b * form a plane perpendicular to L *, a * coordinate corresponds to red if a *> 0, or green if a * <0. The b * coordinate corresponds to yellow if b *> 0, and blue if b * <0.

3.3.3 pH Determination

pH was determined using a digital potentiometer pH-meter brand Conductronic model pH10, previously calibrated, using buffer solutions (pH = 4 and pH = 7), 6 g of fruit were taken and homogenized with 10 mL distilled water. All measurements were carried out in triplicate and result was expressed as average value of determinations.

3.3.4 Preliminary treatment of Acachul fruit

Acachul fruit *(Ardisia Compressa)* was previously washed and sanitized using chlorine at 100 ppm, forthcoming seed was manually separated from the pulp. Subsequently, quantity to be occupied was weighed on a precision electronic balance. To obtain juice of acachul fruit, a Hamilton Beach juice extractor model: 67608 was used, to which 10% (w / v) of encapsulating agents were incorporated with respect to the amount of juice obtained, for which Three samples were prepared with two different biopolymers (Gum arabic brand Fabpsa and Maltodextrin 10D Meyer) and a combination of both in relation (1: 1), incorporation of encapsulating agents was carried out slowly by stirring, using an electronic stirrer model: 102 at 7500 rpm speed following by a vacuum filtration, using a kitasato flask, filter paper, Buchner funnel, vacuum pump, to eliminate the larger pulp residues that could clog nozzles of spray dryer atomizer

3.4 Microencapsulation by spray drying

To encapsulate desired active ingredient (natural acachul pigments), different biopolymeric materials were used as barrier materials, in different proportions, having three treatments as mentioned below in Table 10.1.

Table 10.1 Encapsulating agent used in experimentation

Treatment	Encapsulating agent	Proportion tested
A	Maltodextrin	10% weigth in volume
B	Arabic Gum	10% weigth in volume
C	Arabic Gum- Maltodextrin	10% weigth in volume in proportion 1:1

For spray drying, a Prendo brand spray dryer with serial number 1212IA900054A (Mexico) was utilized, which uses a parallel flow pressure nozzle atomizer. Using different encapsulating agents as barrier materials, such as maltodextrin, arabic gum, and a combination of maltodextrin and arabic gum. Samples were placed for feeding through a peristaltic pump, where drying consists of atomizing the juice generating small droplets, which are microencapsulated by a hot air stream. Being independent variables to control the inlet temperature (140 ° C), feed flow of the sample (3 mL / min), air flow of 10 l / min at a pressure of 5 mm Hg. At the moment in which the drops of acachul juice come into contact with hot air, temperature balance and partial vapor pressure between liquid and gas phases is established. Therefore heat transfer takes place from air to the product as a result of temperature difference while water transfer takes place in the opposite direction due to vapor pressure difference, after that, droplets evaporation is carried out at constant temperature and at partial pressure of water vapor. When water content of droplet reaches a critical value, a dry crust forms on droplet surface, drying rate decreases rapidly and becomes dependent on rate of water diffusion through crust. Drying is theoretically finished when particle temperature is the same as that of the air, passing encapsulated product to a collector. Product (powder) dried by spraying and encapsulated by using different encapsulating agents, was stored in amber vials incorporating N2, which provides an inert atmosphere to avoid reactions of biological material during storage (figure 10.1).

Figure 10.1 Microencapsulation by spray drying

3.5 Characterization of natural pigment

3.5.1 Determination of water activity (a_w)

a_w was determined in an AquaLab equipment, model series 3TE, determination consisted of placing pigment in sample tray, introducing sample and then turning knob to the left to place it in reading position, afterwards withdrawing sample when equipment alarm activates with an intermittent sound.

3.5.2 Water content determination

Water content was determined in an Ohaus brand thermobalance, model MB45 which uses halogen as a heating source, equipment works on the basis of thermogravimetric principle: at the beginning of measurement, moisture analyzer determines sample weight, sample is heated by means of the halogen drying unit and moisture evaporates. During drying operation equipment continuously determines sample weight and presents the result expressed in % of moisture content. Therefore, 0.5 g of sample were placed reaching a temperature of 150 ° C for determination.

3.5.3 Color determination

Color was determined in a Hunterlab model D25A-9000 colorimeter, which was previously calibrated. From the reflection spectra, color coordinates of CIELab system were obtained, each color reading represents the average of three readings made L *, a * and b *. Subsequently, with values obtained from the coordinates, color index (IC *) is determined as a reference of tonality acquired by the simple.

3.5.4 Scanning electron microscopy (SEM)

To evaluate the quality of encapsulated product and the shape of particles obtained, a Jeol JSM-5900LV brand scanning electron microscope, Scanning Electron Microscopy, was used. Samples were placed in sample holder with the help of double-sided carbon tape.
However, as it is a material of biological nature, it was coated with gold (Au) under vacuum in a DENTON VACUUM model DESK V equipment, which gives conductive property to samples, they are protected from collapse by vacuum generated and breakage by the incidence of electrons beam. Particles were studied at magnifications ranging from 1000X to 20,000X.

3.5.5 Determination of total anthocyanins by spectrophotometry

To evaluate the total anthocyanin content of Acachul *(Ardisia compressa)* in all three treatments (a, b and c), a Pharo Merck spectrophotometer was used, scanning at a wavelength from 200 to 600 nm range in which anthocyanins are identified. . Pigment content was calculated as cyanidin-3-glucoside, using an extinction coefficient of 26900 and molecular weight of 449.2, using formula (1) for anthocyanidin quantification.

$$AT\left(\frac{mg}{100g}\right) = \frac{A \cdot PM \cdot FD \cdot 1000}{w \cdot \epsilon} \quad (1)$$

Where: A: is the absorbance; MW: molecular weight; FD: dilution factor; w: sample weight occupied; E: molar extinction coefficient; AT: total anthocyanins expressed in milligrams per 100 grams of fresh fruit.

For samples preparation, a solution of methanol: water: formic acid (70: 28: 2) was used for anthocyanins extraction.

Procedure consisted of weighing 1 g of pigment and depositing it in a mortar, subsequently, 30 mL of acidified methanol was added for further extraction and solubilization, thus obtaining a mother solution. From this solution, 1mL is taken which is added again to 9 mL of acidified methanol solution as well as FD 10 (this was performed for all three treatments), making a scan of 200-600 nm, using distilled water as a blank.

3.5.6 Hygroscopicity determination

Closed systems were used with saturated solutions of different salts that generate different relative humidities at a temperature of 20 ° C, according to what was described in 1980 by Rockland and Nishi. Procedure was carried out for three different treatments, which consisted of placing 0.2 g of sample obtained from each treatment in closed desiccators. Thereafter, closed containers were stored during six days in an extraction hood at 22 ° C temperature and 88% relative humidity, determining the value of water activity (a_w) before and after established time.

Table 10.2 Relative humidity generated by different salts used to determine hygroscopicity at 20 ° C.

Salt	Relative Humity (%) at 20°C
Sodium Hidroxide	8.9
Magnesium Cloride	33.0
Sodium Cloride	75.5
Potassium Sulfate	97.5

3.5.7 Density determination

The method was follow through as described by Papadakis (2006), using a Denver Instrument model TP-214 digital scale (previously calibrated), a 10 mL cylinder, xylene and the sample. Methodology that will be described below was carried out on three treatments. 0.5g of xylene was placed in a 10 mL graduated cylinder, then 0.2 of the pigment was added, this is tapped lightly on work table 10 times at a height of 10 cm. Once the solute has been completely diluted, density is determined. Density is calculated by dividing powder mass between final volume occupied in test tube, formula (2).

$$D = {}^{m}/_{v}. \quad (2)$$

Where: D = density; m: mass; v: volume.

3.5.8 Reconstitution Test

To maintain the efficiency of spray drying process, it is important to ensure that final product will have a rapid and complete rehydration in different media, be it aqueous or in some other type of solvent. For reconstitution or rehydration it involves four conditions: ability to moisturize, ability to submerge, agility to disperse into independent particles in solvent, solubilization. For these conditions, morphology of particles plays an important role in terms of rehydration capacity in spray-dried products. Particles have less rough surfaces and greater sphericity, which gives a smaller surface for water absorption, compared to products that present greater roughness and therefore greater surface available for water absorption. Reconstitution test was carried out using a beaker with a 120 mL aqueous solution (H_2O), and 2 g of pigment (powder), at temperatures of 25, 30, 50 and 80 ° C, determining precipitation time of powder in water. This measurement will indicate the time required to wet samples of natural pigment powder in its entirety. Determination of immersion time was made by using a Casio brand digital chronometer (Model 1121. Manufactured in Mexico).

4 Results

4.1 Physicochemical characterization of fruit and encapsulated pigment

Table 10.3 shows average values and standard deviation of pH, soluble solid content (°Bx) moisture content (%) of fresh acachul fruit *(Ardisia compressa)* stored at a refrigeration temperature of -4 °C

Table 10.3 Physicochemical characterization of Acachul *(Ardisia compressa)*

pH	ºBrix	Moisture Content (%) (%)
3.48 ± 0.72	7.8 ± 0.3	77.45± 2.92

4.2 Characterization of Acachul pigment *(Ardisia compressa)* obtained by spray drying

Table 4 shows water activity and moisture content (%) of powdered acachul fruit once spray drying has been carried out, using an inlet air temperature of 140 ° C, with a feed of (3 mL / min) and 5mm Hg air pressure. Results indicate that regardless of polymeric material used to encapsulate the product, acachul is a product with a water activity of around (0.348 ± 0.004), and a variable moisture content depending on the encapsulating agent to be used. So it can be said that stability of encapsulated pigment depends on the encapsulating agent to be used. As can be seen in Table 10.4, it is observed that treatment C when using maltodextrin as an encapsulating agent, moisture content of product is 7.8%, a moisture content lower than that obtained when using treatment A or B.

Furthermore, it is considered that the product encapsulated with maltodextrin, has a lower proliferation capacity of deteriorative microorganisms compared to other treatments.

Table 10.4 Results of Water Activity (a_w) and moisture (%) of powdered acachul fruit

Treatment	(a_w)	Moisture Content (%)
A	0.364 ± 0.009	12.01 ± 0.14
B	0.323 ± 0.004	14.41 ± 0.39
C	0.358 ± 0.004	07.80 ± 0.27

4.2.1 Color determination

Cielab color parameters (L, a, b) of powdered acachul obtained from spray drying, when using different encapsulating agents, are presented in Table 10.5, being these parameters the following.

Table 10.5 Color parameters of CIELab system and color index (IC *) obtained from the treatments (A, B and C) obtained by spray drying

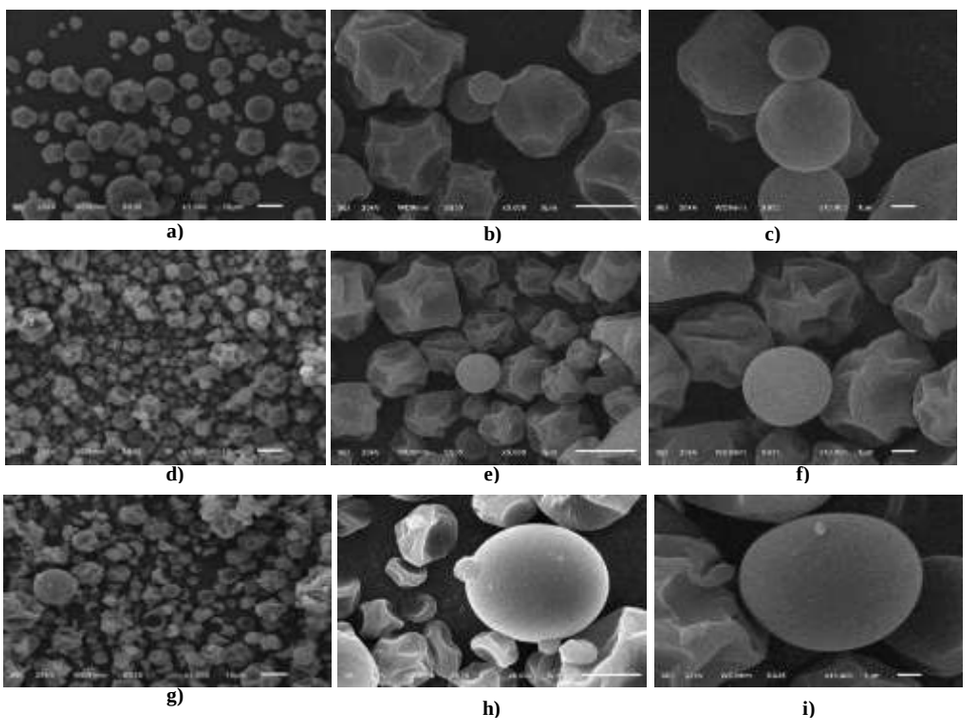
Colorimetry	Treatment		
	A	B	C
L*	43.02 ± 0.02	43.12 ± 0.01	38.82 ± 0.01
a*	31.40 ± 0.03	29.52 ± 0.03	30.86 ± 0.1
b*	-3.78 ± 0.06	-4.68 ± 0.09	-3.80 ± 0.08
IC*	-192.94 ± 3.5	-146.18 ± 2.94	-209.80 ± 3.77

4.2.2 Scanning electron microscopy (SEM) of powders

In figure 10.2, morphology of microcapsules can be observed in treatments (A, B and C) that were used to microencapsulate anthocyanins at a concentration of 10% at a temperature of 140 ° C. Microcapsules are of variable size and shape, that is to say, there are capsules with a smooth spherical shape and capsules with a dented or irregular surface. Variation in particle size ranges from 2,209 µm to 21,715 µm. Even in the size variation it was not isotropic.

Figure 10.2 Scanning electron microscopy photomicrographs of acachul pigment microcapsules from Acachul *(Ardisia Compressa),* obtained by spray drying for the different treatments.

Treatment A **a)**1000x **b)** 5000x **c)** 10000x
Treatment B **d)** 1000x **e)** 5000x **f)** 10000x
Treatment C **g)** 1000x **h)** 5000x **i)** 10000x



Treatments B and C present spherical particles with smooth surfaces and semi-uniform sizes, observing anisotropic shapes, that is, irregular shapes on the surface, this behavior is due to the fact that particles present deformation and fracture in their structure, attributable to vacuum generated in scanning electron microscope observation chamber for electron beam scanning. Formation of dents on capsule surface can be attributed to contraction of particles during drying, due to drastic loss of moisture followed by cooling. In general, for three treatments carried out, it is observed that particles do not reach a size at the nanometric level, since there are particles with size greater than 1 µm.

4.2.3 Determination of total anthocyanins (TA)

Determination of total anthocyanins was accomplish at (λ) 523nm wavelength. Wavelength that presents response at 523 nm corresponding to cyanidin-3-glucoside (Total anthocyanin content) expressed in mg per 100 g of fresh fruit. Results of absorbance and total anthocyanins (AT) for fresh fruit and treatments A, B and C, are presented in Table 10.6.

Table 10.6 Absorbance results (A) obtained by spectrophotometry to determine total content of anthocyanins (AT)

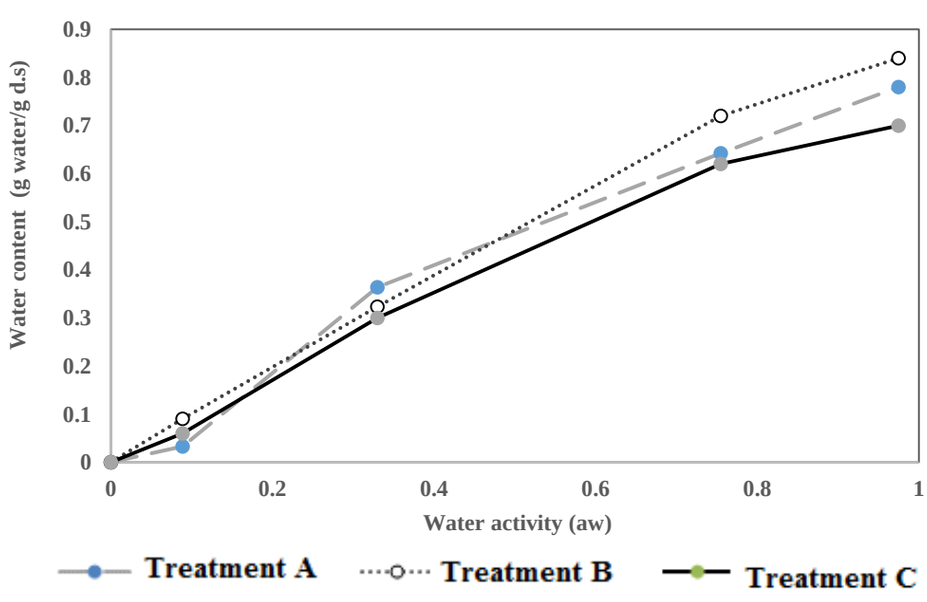
Treatment	λ nm	A	AT (mg/100 g)
A	523	0.4434	82.76
B	523	0.4986	93.57
C	523	0.3232	60.62
Fresh fruit	523	0.8392	157.53

4.2.4. Hygroscopicity

A measure of the degree of hydration of the sample under different moisture content atmospheres is the determination of the hygroscopicity under different atmospheres of relative moisture content, represented by isotherms of the different encapsulation treatments at storage temperature at 20 ° C. As can be seen in graphic 10.1, it is observed that treatment

A presents a higher hygroscopicity condition than treatments B and C, a behavior that is attributable to the fact that in treatment B (Encapsulated with Gum Arabic), water molecules intertwine with product components due to formation of hydrogen bonds in competition with water solute bonds due to the fact that the polar groups –OH and H behave as active sorption centers. According to results obtained, it could be considered that the product should be kept at a relative moisture content of less than 33% at room temperature to prevent deterioration reactions and prevent the product from hydrating, showing the appearance of wet powder or with agglomerate formation characteristics due to hydration of powdered product, which will affect its stability and reduce its shelf life significantly.

Graphic 10.1



4.2.5. Reconstitution

Reconstitution time of dehydrated powder pigment in an aqueous medium to determine the behavior of the dehydrated product when it is subjected to rehydration with water, was carried out at rehydration water temperatures of 25, 30, 50 and 80 ° C, results that are presented in Table 10.7.

Table 10.7 Pigment reconstitution time obtained by spray drying with different encapsulating agents

Temperature (ºC)	Treatment A	Treatment B	Treatment C
25	24	24	27
30	22	23	26
50	21	22	23
80	9	21	14

As can be seen from Table 10.7, it is observed that time to effect the dissolution of the powdered pigment is inversely proportional to time used in the reconstitution. This behavior is attributable to the fact that surface tension of water decreases with temperature, since the cohesion forces decrease with increasing water temperature. Influence of the external environment is due to the fact that the molecules of the environment exert attractive actions on the molecules located on the surface of the liquid, counteracting the actions of the molecules of the liquid.

4.2.6 Apparent density

Apparent density of obtained product is the ratio of mass and its volume, including the volume contribution of the empty space between particles. Consequently, apparent density depends on both density of the powder particles and the spatial distribution of particles in the powder bed. Properties that determine apparent density of powdered pigment depend on preparation, encapsulating agent used and sample storage, that is to say on the way of handling. Particles can be compacted to have a variable range of apparent densities; however, slightest disturbance to powder bed can cause a change in apparent density. Obtained results are presented in Table 10.8.

Table 10.8 Apparent density of powder pigment when using different encapsulating agents

Treatment	Apparent density (g/ml)
A	1
B	1
C	0.5

From the above table it can be observed that density of encapsulated powder with maltodextrin: arabic gum, has a lower density than when using other encapsulating agents, behavior attributable to the occurrence that empty space between particles is less than when using other encapsulating agents.

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Conclusions

Anthocyanins, although they can be recovered from various types of fruits and vegetables, commonly grapes, strawberries, black currants, purple cabbage and others, for economic reasons, are obtained mainly from by-products derived from wine industry, especially the grape peel, therewithal it can be considered that acachul can be a good alternative to obtain these pigments. Analysis of acachul anthocyanin content, reveal to have a significantly high anthocyanin content compared to that reported for other fruits and by other authors, which can make acachul an important source for obtaining this type of pigments, especially to obtain extracts with potential for application as food colorants.

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