Vol. 18, No. 3 (2019) 1261-1276
Revista Mexicana de Ingeniería Química

MICROENCAPSULATION OF BIOCOMPOUNDS FROM AVOCADO LEAVES OILY EXTRACTS

MICROENCAPSULACÍÓN DE COMPUESTOS BIOACTIVOS DE EXTRACTOS OLEOSOS DE HOJAS DE AGUACATE

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Received: November 23, 2018; Accepted:

Abstract

Microencapsulation of natural biocompounds is a growing field leading to its incorporation into food formulations. The use of vegetable oils as alternative solvents to extract biocompounds is considered a green process. Although avocado leaves are underexploited they are rich in naturally-occuring biocompounds. Therefore, we propose the extraction of biocompunds from avocado leaves using edible vegetable oils and preserve them by means of spray-drying microencapsulation. Spray drying allowed an encapsulation efficiency of the avocado leaves pigments of 50%, extracted with edible oils. Microcapsules Haussner ratio values (1.49 - 1.61) showed the cohesiveness of the powders, this was confirmed by microscopy techniques, obtaining high particles area values (177.4 - 4460.5 μm^2). Corn oil extracts showed higher carotenoids (>10%), chlorophyll a (approximately 5%) and chlorophyll b (>40%) content as compared with safflower oil extracts. However, the hygroscopicity values of the corn oil microcapsules (15.70 - 18.34 g water/ 100 g dry capsules) caused the dissolution of the wall materials exposing the microencapsulated oil, changing the sample color from white to pale yellow.

Keywords: Avocado leaves, oily extracts, spray drying, microencapsulation, antioxidants.

Resumen

La microencapsulación de biocompuestos para su incorporación en alimentos, es un área en desarrollo. El uso de aceites vegetales como disolventes para la extracción de bioactivos, es considerado un proceso verde. Las hojas de aguacate son poco explotadas y contienen de forma natural biocompuestos. Por esto, se propone la extracción de biocompuestos de hojas de aguacate utilizando aceites vegetales como disolventes y su posterior microencapsulación utilizando secado por aspersión. El secado por aspersión tuvo una eficiencia de encapsulación del 50% de los pigmentos extraídos. Los valores del Índice de Haussner demuestran la cohesividad de las mismas (1.49 - 1.61), esto se confirmó obteniendo el área de las partículas (177.4 - 4460.5 mum^2) usando microscopía. Los extractos con aceite de maíz tuvieron mayor contenido de carotenoides (>10%), clorofila a (aproximadamente 5%) y clorofila b (>40%), comparado con los extractos de aceite de cártamo. Sin embargo, debido a la elevada higroscopicidad de las microcápsulas de extractos con aceite de maíz (15.70 - 18.34 g agua / 100 g cápsulas base seca) los materiales pared se disuelven, liberando al aceite encapsulado en el interior, cambiando las muestras de color de blanco a amarillo.

Palabras clave: Hojas de aguacate, extractos oleosos, secado por aspersión, microencapsulación, antioxidantes.

1 Introduction

Microencapsulation is a technology in which small particles containing valuable core substances embedded by a wall material are subjected to dehydration to produce capsules in which the core material is protected from deterioration due to external factors. The microencapsulation of natural bio compounds is a growing field leading to its incorporation into food formulations (Böger *et al*., 2018; Guadarrama-Lezama *et al*., 2012). Spray drying is one of the most used microencapsulation methods, given its suitability for different core materials, such as hydroalcoholic extracts, oleoresins and oils, producing high-quality microcapsules, with a particle size of less than 40 μm (Dias *et al*., 2015).

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Tel. 57-29-60-00 Ext 57831
https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Plazola
issn-e: 2395-8472

The first step in the microencapsulation of oily core substances is to prepare a stable emulsion, by mixing the core material and the polymers used as wall materials and subjecting the mix to high-pressure homogenization/microfluidization/ultrasound/rotor-stator high shear mixer among others (Bae and Lee, 2008; Fuentes-Ortega *et al*., 2017). Carotenoids and chlorophylls are some of the most abundant liposoluble biocompounds and frequently, hydrocarbon-non-polar solvents produced in the petrochemical industry and which are toxic for the environment and for the human being are used for the extraction of these compounds (Derrien *et al*., 2018; Ordóñez-Santos *et al*., 2015). The use of these solvents require complex and costly steps for their separation/recovery. The use of vegetable oils as promising alternative solvents to extract these bioactive compounds may be considered as part of a green processes since they are considered environmentally friendly solvents (Goula *et al*., 2017). Moreover, oil can act as a barrier against oxygen thus, retarding oxidation time and extent of degradation of the extracted biocompounds (Goula *et al*., 2017). Kang *et al*. (2019) use edible oil as solvent for chlorophyll and lutein extracted from spinach by-products; however the extraction was carried out using acetone. Oil has demonstrated to be adequate solvents for the extraction of carotenoids from different by-products, such as shrimp (Sachindra and Mahendrakar, 2005), carrot (Li *et al*., 2013), peach palm fruit (Ordóñez-Santos *et al*., 2015) and pomegranate (Goula *et al*., 2017).

Persea americana commonly known as avocado belongs to the *Lauraceae* family which is native of Mexico and Central America and currently cultivated in most tropical and subtropical countries of the world. Avocado is a medium-sized, single-stemmed, erect, perennial and deciduous tree of 15-20 m in height (Musabayane *et al*., 2007; Ojewole and Amabeoku, 2006). Avocado is an economically important crop due to its nutritional value; however, the other three constituents such as fruit peel, seed and leaves are underexploited.

Avocado trees have their first harvest after 2 or 5 years, however during this time avocado leaves are continuously produced (Araújo *et al*., 2018). Avocado leaves are industrially underexploited and are considered a bio-waste in spite of being rich in naturally-occurring bioactive compounds, such as luteolin, rutin, orhamnetin, quercetin and apigenine, caffeic, chlorogenic, coumaric, ferulic, gallic, hydroxybenzoic, protocatechuic, pyrocatechuic, resorcylic, sinapic, syringic and vanillic acids, catechin and epicatechin, which can act as coadjutants in treatment of diseases related to oxidative stress (Duarte *et al*., 2016; Jiménez *et al*., 2017; Soquetta *et al*., 2018). It has been reported that infusions or hydroalcoholic extracts from avocado leaves are used in traditional medicine, and have been catalogued to have pharmacological activities, being some of them analgesic, diuretic, antidiabetic, hypoglycemic, anti-inflammatory and anti-diarrheal (Duarte *et al*., 2016; Musabayane *et al*., 2007; Yamassaki *et al*., 2017).

No studies have been published on the use of vegetable oils to extract biocompounds from avocado leaves and, considering the availability of large quantities of such leaves and their potential as a renewable feedstock for the production of intermediate value-added compounds, the aim of this work was to use edible oils to extract biocompounds as chlorophylls and carotenoids from avocado leaves and the preservation of the oily extracts by means of spray drying microencapsulation.

2 Materials and methods

2.1 Plant material

The avocado (*P. americana*) leaves from *Hass* and *drymifolia* (Creole) varieties were purchased on a local market on April 2018. After sanitization, the leaves were lyophilized for 8 hours in a freeze dryer (Labconco, USA). Dried leaves were grinded and sieved by using a 50 mesh (< 297 μm). The powder was stored under vacuum in plastic bags protected from light in a desiccator at room temperature.

2.2 Chemical reagents

Commercial corn (C) and safflower oil (S) were purchased in a local supermarket in Mexico City. Gum arabic (GA) and maltodextrin (MD) were used as wall materials. All chemicals used in this work were reagent grade. Hexane, isopropanol, petroleum ether, acetic acid, sodium acetate, KH_2PO_4 and Na_2HPO_4 were purchased from J.T Baker, acetone and $FeCl_3{\cdot}6H_2O$ were purchased from Fermont. ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate, Trolox ((~)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) were purchased from Sigma Aldrich.

2.3 Extraction

The extraction was performed by using the above described vegetable oils which were mixed with the avocado leaves powders from *Hass* (H) and *drymifolia* (A) varieties at a 1:100 (w/w) ratio (Kang *et al*., 2019). The extraction was carried out by mechanical agitation (Super Nuova SP135935, Waltham, MA, USA) of the mixtures at 700 rpm for 6 hours at room temperature. Then, the oil extracts were filter through a Whatman filter No. 1 and centrifuged (Hermle Z326K, Wehingen, Germany) at 3700 rpm for 15 min at 10 °C. Finally, the extracts were stored at 4 °C in amber glass bottles until further use. The oil extracts obtained were named as follow: HS *Hass* avocado leaves extracted with safflower oil, AS Creole avocado leaves extracted with safflower oil, HC *Hass* avocado leaves extracted with corn oil and AC Creole avocado leaves extracted with corn oil.

2.4 Emulsion preparation

An emulsion, oil and water (o/w), was prepared by following the method described by Guadarrama-Lezama *et al*. (2012). Each oil extract was mixed with a wall materials solution (10 g of gum arabic and 10 g of maltodextrin dissolved in 100 mL of distilled water and stored overnight for total rehydration) in a ratio of 1:4 (w/w). The extracts were added drop-by-drop to the solution of biopolymers while homogenizing by using a high-speed disperser (Ultra-Turrax, M45, USA) at 11,000 rpm. After the addition of the extracts, the emulsions were homogenized for 3 additional minutes (Guadarrama-Lezama *et al*., 2012; Pereyra-Castro *et al*., 2018)

2.5 Particle size and Z-potential

Immediately after the homogenization, the particle sizes of the emulsions and the polydispersity index (PDI) were evaluated by Dinamic Light Scattering, and the Z-potential was evaluated by Laser Dopller Microelectrophoresis. These measurements were carried out by using a particle analyzer (Zetasizer NANO-S90, Malvern Instruments Limited, Worcestershire, UK). The sample was diluted (1:100) with distilled water to avoid multiple scattering effects and one milliliter of the diluted sample was added to the cuvette prior to be inserted in the equipment (Pereyra-Castro *et al*., 2019).

2.6 Spray drying

A Mobile Minor™ 2000 spray dryer (GEA Niro, Denmark) was used to conduct the drying of the emulsions which were feed in a parallel flow, respect to the airflow to the drier chamber by using a peristaltic pump (Watson-Marlow 520S, USA) at a rate of 20 mL/min. The inlet and outlet temperatures of the drying air were 180 and 80 °C respectively, and the air pressure of atomization was 1 kg/cm^2. The obtained powders were stored into plastic bags, hermetically sealed, at room temperature in the absence of light and inside a desiccator, until characterization analyses were performed with the exception of the evaluation of moisture content and water activity which were determined immediately after drying (Pereyra-Castro *et al*., 2018). The powders were named as follow: HSM *Hass* avocado safflower oil extract microcapsules; ASM creole avocado safflower oil extract microcapsules; HCM *Hass* avocado corn oil microcapsules; ACM creole avocado corn oil microcapsules.

2.7 Microcapsules characterization

2.7.1 Encapsulation efficiency, total and surface oil

Surface oil was calculated by using the method reported by Kang *et al*. (2019). Two grams of microcapsules were dissolved in 3 mL of distilled water at 50 °C by stirring in a vortex for 3 minutes after which, 10 mL of a hexane-isopropanol (3:1) mixture were added, stirred for 5 minutes and centrifuged at 3600 rpm for 15 min at 10 °C. The organic phase was separated and then placed into a 50 mL round-bottom flask, and the solvent was evaporated by using a rotary vacuum evaporator (HahnShin, Model HS-2000NS, South Korea) until constant weight. The surface oil was measured by mixing 0.5 g of each powder with 20 mL of petroleum ether and shaken for 15 minutes at room temperature. After, the mixture was filtered and the solvent was removed using a rotary vacuum evaporator until constant weight.

The encapsulation efficiency (%EE) was calculated with Eq. (1), as a relation between the surface oil (So) and the total oil (To) (Böger *et al*., 2018).

$$\%EE = ((To - So)/To) \times 100 \quad (1)$$

2.7.2 *Moisture content and water activity*

Moisture content was measured by using a gravimetric method (AOAC, 1995). Briefly, 1 g of each powder was placed in an aluminum plate at constant weight and heated at 105 °C until constant weight. Water activity was measured by means of an Aqualab meter, Decagon Devices, Model 4 TE, USA.

2.7.3 *Flow properties*

The bulk and taped density results were obtained from the relation between the material weight and the volume that occupied without packing and after packing respectively. For determining both parameters, two grams of each sample were placed in a 10 mL test tube. Bulk density was registered as the volume occupied by the sample without packing, and the packed density was registered after tapping the test tube and reaching a constant volume value. Haussner ratio was calculated as the relation between the tapped and the bulk density (Pereyra-Castro *et al*., 2018).

2.7.4 *Hygroscopicity*

The hygroscopicity was determined according to the method reported by Pereyra-Castro *et al*. (2018) in which 1 g of each sample was placed in a container with a saturated NaCl solution (75.29% of relativity humidity at 25 °C). After a week the samples were weighted and the difference between final and initial weight was expressed as g H_2O / 100 g of dry solids.

2.7.5 *Dissolution*

The dissolution of the powders was measured according to the spectrophotometric method described by Tang and Li (2013). In a cuvette 3 milliliters of distilled water were added and 30 mg of powder sample were layered on top. The changes in the absorbance of the solution at 620 nm were recorded to evaluate the rate of dissolution given by the k_0 as described in section 3.3.6.

2.7.6 *Morphometry of microparticles*

Image acquisition was performed manually by using a light microscope (CILAS 1090-ExpertShape-NT 2107380, France), which has a video camera with a peak bandwidth of 23.2 MB/s. Morphological parameters were analyzed by using the ExpertShape software. The morphological parameters measured were area (A), perimeter (P), mean Feret diameter, maximum and minimum Feret diameters, equivalent diameter, roundness and the equivalent ellipse ratio (Jiménez-Guzmán *et al*., 2016). Also, scanning electron microscopy was used to observe morphology and surface characteristics of the microparticles. Samples were examined at 200x, 1500x and 3000x in a scanning electron microscope (JSM 5800LV, Jeol Inc., USA) at 10^{-7} mbar at 15 KV, equipped with a digital image capture software.

2.7.7 *Pigment quantification*

The pigment quantification (carotenoids and chlorophylls) was carried out for the oil extracts and for the microcapsules using a spectrophotometer UV-VIS (Jenway 6705, Staffordshire, UK) according to Brahmi *et al*. (2013) and Lichtenthaler and Buschmann (2001) . Briefly 300 mg of the extract were mixed with 4000 μL of acetone and filtered through a syringe filter. The chlorophyll a (C_a) and chlorophyll b (C_b), and the total carotenoids C_t concentrations were calculated with the Eq. (2) - (4) and expressed as μg/mL:

$$C_a = 11.24A_{661.6nm} - 2.04A_{644.8nm} \quad (2)$$

$$C_b = 20.13A_{644.8nm} - 4.19A_{661.6nm} \quad (3)$$

$$Ct = (1000A_{470nm} - 1.90C_a - 63.14C_b)/214 \quad (4)$$

A control extraction using hexane as dissolvent was performed on *Hass* and Creole avocado leaves. After solvent evaporation, the extracts were re-dissolved with acetone and the pigments were quantified. Corn an safflower commercial oils, contain pigments naturally due to their vegetable origin, in order to only quantified pigments extracted from avocado leaves, for each determination a control sample of corn or safflower oil was carried out. This value was subtracted to the sample quantification for each pigment.

2.7.8 *Trolox equivalent antioxidant capacity (TEAC) assay*

The TEAC method was used and the stable radical $ABTS^{\cdot+}$ radical reagent was obtained by mixing 2.5 mL of stock solution of ABTS (7 mM) with 44 μL of stock solution of potassium persulfate (2.45 mM). This solution was left to react for 16 h in the dark at room temperature. After 16 h, 1 mL of the mixture was diluted with 70 mL of 5 mM PBS (pH 7.4) and

adjusted to and absorbance of 0.7 at 734 nm. One milliliter of this ABTS solution was mixed with 10 μL of each sample and the absorbance measured after 45 minutes. The results were presented as μM TE / g of oil using a Trolox standard curve (0 - 2000 μM) (Pellegrini *et al*., 2003). A control sample was carried out in order to eliminate the antioxidant activity of antioxidants contained in vegetable commercial oils.

2.7.9 *FRAP method*

This method was carried out according to Benzie and Strain (1999). Three solutions were prepared: the first one was: 300 mM acetate buffer with a pH 3.6 (solution A), the second was TPTZ 10 mM in HCl 40 mM (solution B) and the third one was $FeCl_3 \cdot 6H_2O$ 20 mM (solution C). The FRAP work solution was prepared by mixing 25 mL of the solution A with 2.5 ml of the solution B and 2.5 mL of the solution C. Three milliliters of the FRAP solution were mixed with 100 μL of each sample and incubated at 37 °C for 30 minutes. After this time, the absorbance at 593 nm was recorded. The results were presented as μM TE/g of oil using a Trolox standard curve (0 - 1500 μM) (Benzie and Strain, 1999; Pellegrini *et al*., 2003). A control sample was carried out in order to eliminate the antioxidant activity of antioxidants contained in vegetable commercial oils.

2.8 *Statistical analysis*

All the results were expressed as the mean values of tree replicates ± the standard deviation. The analysis of the results among samples were analyzed with a one-way ANOVA test, significant differences were determined at 95% confidence by Tukey's test by using GraphPad Prism software v. 5.0 (CA, USA, 2015).

3 Results and discussion

3.1 *Emulsion micelles size and Z-potential*

The emulsion formed for the spray-drying microencapsulation must have a small size and be stable to agglomeration (Bae and Lee, 2008). In Table 1, it is possible to observe that the type of oil and leaf origin, did not impact significantly ($p < 0.05$) to the emulsions droplet size.

The range of particle sizes obtained was 2.6 to 3.8 μm. These values are lower than those reported by (Böger *et al*., 2018) who used the same wall materials (GA and MD) for the encapsulation of grape seed oil and obtained an emulsion droplet size of 5.80 ± 0.11 μm. The difference could be due to the relation between wall materials-oil (1:9) and the homogenization conditions applied to produce the emulsions (16000 rpm / 5 min).

Avocado leaves oil extracts emulsions had a heterogeneous particle size distribution since Polydispersity index (PDI) obtained varied from 0.5 to 0.87. Polydispesity index (PDI) is a dimensionless parameter which indicates the distribution amplitude of the droplet sizes formed into the emulsion, this parameter can have values between 0 and 1. A 0 value shows that the emulsion is perfectly uniform and the value increases as the heterogeneity of the particle size of the emulsion increase (Ricaurte *et al*., 2016; Shamaei *et al*., 2017; Villalobos-Castillejos *et al*., 2017).

Although Zeta -potential values of the emulsions prepared with corn oil extract are slightly lower than those corresponding to the other samples there were not significantly different among them ($p > 0.05$). Zeta-potential is the measure of the electro kinetic charge between the drop and the dispersing media.

Table 1. Micelles size and zeta potential of the emulsions.

Sample	d (μm)	PDI	ZP (mV)
HSE	2.97 ± 0.67^a	0.64 ± 0.16^a	-35.9 ± 1.76^a
ASE	2.63 ± 1.34^a	0.87 ± 0.08^a	-35.9 ± 1.06^a
HCE	3.81 ± 1.62^a	0.76 ± 0.27^a	-38.0 ± 0.74^b
ACE	3.74 ± 1.72^a	0.52 ± 0.28^b	-37.2 ± 1.24^a

HSE: Hass avocado safflower oil extract emulsion; ASE: creole avocado safflower oil extract emulsion; HCE: Hass avocado corn oil extract emulsion; ACE: creole avocado corn oil extract emulsion.

^{a,b} Different letters on the same column shows significant difference ($p < 0.05$).

To consider an emulsion as stable its Z-potential must be higher than |30 mV| (Ricaurte *et al*., 2016). The prepared emulsions are electro kinetically stable since the Zeta-potential values obtained were lower than -30 mV.

3.2 Microcapsules characterization

3.2.1 Encapsulation efficiency, total and surface oil

Table 2, shows the encapsulation efficiency and the total and surface oil of microencapsulates of the oil extracts. The results showed that oil used as solvent did not have a direct impact on the encapsulation efficiency and did not show significant difference ($p < 0.05$) among the samples. The encapsulation efficiency of the oil extracts was approximately 50%, which means that 50% of the oil extracts remains inside the microcapsules before the spray drying. This helps preserving the chemical characteristics of the extracted biocompounds. Also, the surface oil has a direct impact on other properties such as moisture content. The surface oil acts as a barrier to the moisture diffusion from the ambient and thus influencing hygroscopicity (Kang *et al*., 2019).

3.2.2 Moisture content and water activity

Moisture content and water activity are properties that affect powder characteristics such as flowability, caking and have a direct impact on the stability during the storage of the bioactive compounds used as core materials since promote their oxidation (Bhusari *et al*., 2014; Kang *et al*., 2019). Table 3, shows the moisture content of avocado leaves oil extracts powders obtained after spray drying. The moisture content was below 5% (between the range 2.7-4.9%) being HCM the only one that showed significant differences ($p < 0.05$) among the microencapsulated extracts.

These results are similar to those reported for microparticles of curcumin dissolved in coconut oil using GA as wall material (Bucurescu *et al*., 2018), and for microparticles of chlorophyll prepared by using MD and GA as wall materials (Kang *et al*., 2019). Both authors obtained moisture content lower than 2.5%. This value could be associated with the relation between MD and GA since moisture content is inversely related to total solids in the sample. An increment in the wall material concentration, produces lower moisture contents of the capsule (Seerangurayar *et al*., 2017).

Moisture and water activity are related to stability and shelf life of the final product. Moisture content includes free and bound water, whereas water activity is the measure of the free water available for biochemical reactions leading to degradation (Seerangurayar *et al*., 2017). The results of moisture content and water activity of the microencapsulated oily extracts (Table 3) ensure an extended shelf life of the product, reducing the risk of bacteriological development. Thus, these encapsulates are adequate for their use in the food industry, where the recommended values for powders moisture content are between 3 - 4%. (Bucurescu *et al*., 2018; Fuentes-Ortega *et al*., 2017). It is noteworthy that the microencapsulates with higher surface oil contents also presented higher moisture and water activity values which might be due to the surface oil which acted as a barrier avoiding losses of moisture during and after drying (Kang *et al*., 2019).

Table 2. Total and superficial oil and encapsulation efficiency of avocado leaves oily extracts spray dried.

Sample	Total oil (g oil/ g powder d.b.)	Superficial oil (g oil / g powder d.b.)	Encapsulation Efficiency %
HSM	0.12 ± 0.02^a	0.06 ± 0.00^a	51.43
ASM	0.14 ± 0.01^a	0.06 ± 0.00^a	55.42
HCM	0.12 ± 0.01^a	0.06 ± 0.02^a	48.95
ACM	0.12 ± 0.05^a	0.06 ± 0.00^a	48.7

HSM: Hass avocado safflower oil extract microcapsules; ASM: creole avocado safflower oil extract microcapsules; HCM: Hass avocado corn oil extract microcapsules; ACM: creole avocado corn oil extract microcapsules. Total and superficial oil units are g oil / g of powder

[a] Different letters on the same column shows significant difference ($p < 0.05$)

Table 3. Moisture content and water activity of the microcapsules loaded with avocado leaves oily extracts

Sample	Moisture Content %	Water activity
HSM	2.79 ± 0.15^{b}	0.17 ± 0.00^{a}
ASM	2.95 ± 0.57^{b}	0.11 ± 0.00^{b}
HCM	4.99 ± 0.25^{a}	0.21 ± 0.03^{c}
ACM	3.24 ± 0.53^{b}	0.17 ± 0.01^{a}

HSM: Hass avocado safflower oil extract microcapsules; ASM: creole avocado safflower oil extract microcapsules; HCM: Hass avocado corn oil extract microcapsules; ACM: creole avocado corn oil extract microcapsules. ^{a,b,c} Different letters on the same column shows significant difference ($p < 0.05$)

3.2.3 Flow properties

Flowability is a powder property influenced by particle size distribution, moisture content, and angle of repose as well as bulk and tapped density. With the last two parameters it is possible to calculate the compressibility index and Haussner ratio (Seerangurayar *et al*., 2017).

Bulk and tapped density influence the particle packing arrangement and the compaction profile of the powders that can affect their flowability and caking (Pereyra-Castro *et al*., 2018). Table 4 shows the results of the bulk and tapped density as well as the Haussner ratio for the capsules. It is possible to observe that there were not significant differences among the samples (p>0.05).

Some authors have reported that air inlet temperature affects directly the bulk density. Higher values of air inlet temperature produces faster drying rates, resulting larger volumes of the powders due to expansion of particles with low values of bulk density (Alamilla-Beltrán *et al*., 2005; Kalkan *et al*., 2017; Tonon *et al*., 2008). Haussner ratios higher than 1.6, indicate that the powder particles have higher cohesiveness between them (Pereyra-Castro *et al*., 2018). Haussner ratio values of HSM, HCM and ACM were slightly lower than 1.6 while value for ASM was 1.6, indicating that the powders were highly cohesive.

3.2.4 Hygroscopicity

Hygroscopicity is a parameter, which measures the powder capacity to absorb the moisture from the environment and is an important parameter that affects powder flowability and caking. Moreover, hygroscopicity is important when the core material is oil since it is susceptible to lipid oxidation (Pereyra-Castro *et al*., 2018; Saifullah *et al*., 2016). The water absorbed by the avocado leaves oily extracts spray-dried is shown in Table 5. The results showed that capsules are highly hygroscopic, the amount of water absorbed after 7 days was between 13 - 18 g per 100 g of capsules (dry weight basis) and have significant difference among them ($p < 0.05$). These results might be due to GA being a highly hygroscopic material (Toledo Hijo *et al*., 2015).

Table 4. Flowability properties of microencapsulated oily extracts.

Sample	Apparent density (g/cm^3)	Tapped density (g/cm^3)	Haussner ratio
HSM	0.3336 ± 0.01^{a}	0.5274 ± 0.03^{a}	1.5837 ± 0.14^{a}
ASM	0.3258 ± 0.01^{a}	0.5257 ± 0.00^{a}	1.6140 ± 0.03^{a}
HCM	0.3206 ± 0.02^{a}	0.5096 ± 0.01^{a}	1.5921 ± 0.06^{a}
ACM	0.3672 ± 0.02^{b}	0.5472 ± 0.02^{a}	1.4932 ± 0.11^{a}

HSM: Hass avocado safflower oil extract microcapsules; ASM: creole avocado safflower oil extract microcapsules; HCM: Hass avocado corn oil extract microcapsules; ACM: creole avocado corn oil extract microcapsules.

^{a,b} Different letters on the same column shows significant difference ($p < 0.05$)

Table 5. Hygroscopicity of oily avocado leaves microcapsules.

Sample	Higroscopicity (g water/ 100 g dry capsules)
HSM	13.38 ± 0.11^{a}
ASM	14.06 ± 0.12^{b}
HCM	18.34 ± 0.04^{c}
ACM	15.70 ± 0.05^{d}

HSM: Hass avocado safflower oil extract microcapsules; ASM: creole avocado safflower oil extract microcapsules; HCM: Hass avocado corn oil extract microcapsules; ACM: creole avocado corn oil extract microcapsules.

^{a,b} Different letters on the same column shows significant difference ($p < 0.05$)

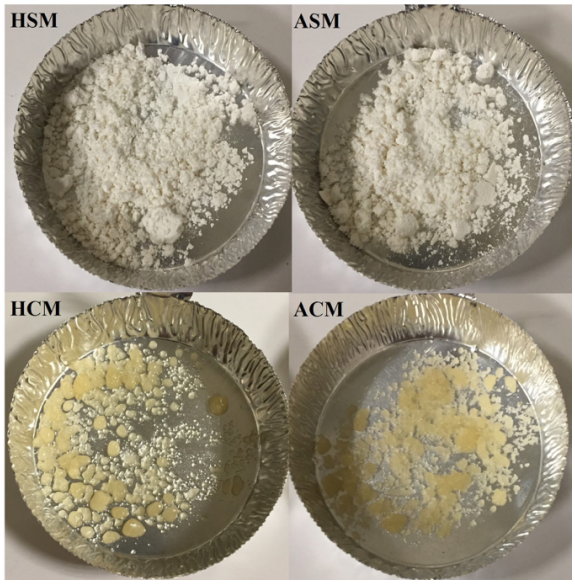

Fig. 1. Hygroscopicity of avocado leaves oily extracts spray dried

As it was mentioned in the encapsulation efficiency section, the absence of surface oil might expose a larger number of sites of water molecules binding the wall materials molecules while the presence of surface oil might help preventing the interaction between GA and water (Kang *et al*., 2019; Pereyra-Castro *et al*., 2018). Comparing the result of Table 2 with those of Table 5, the powders with the lowest encapsulation efficiency also corresponded to the extracts with higher hygroscopicity (HCM and ACM) and were those corresponding to the corn oil extracts. Fig. 1 shows the differences between the hygroscopicity of the extracts using safflower oil (HSM and ASM) and corn oil (HCM and ACM) as solvent. Safflower extracts had the lowest hygroscopicity values and maintained their initial physical characteristics. Meanwhile HCM and ACM microencapsulates changed their appearance, turning their color from white to pale yellow. The particle size might also impact in the hygroscopicity since large particles have also large empty spaces between them, causing that water molecules enter into these empty spaces and interact with hydrophilic molecules on the surface of the powders (Cynthia *et al*., 2015; Pereyra-Castro *et al*., 2018).

3.2.5 *Dissolution*

The increasing values in absorbance during the initial incubation (k_0) showed the powder capacity to be dissolved in water. The final absorbance value reflects the amount of dissolved powder (Pereyra-Castro *et al*., 2018; Tang and Li, 2013). Fig. 2 shows the dissolution behaviors of oil extracts microencapsulates. In Table 6 the parameters k_0 and the final absorbance at 620 nm are presented.

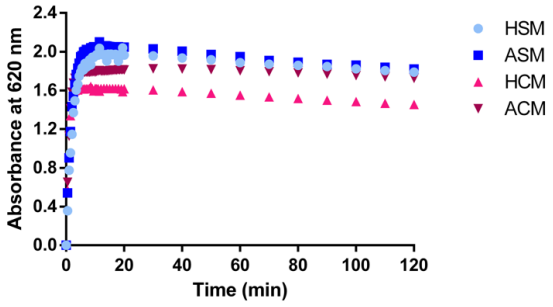

Fig. 2. Dissolution behavior of avocado leaves oily extracts spray dried.

Table 6. Dissolution profiles of avocado leaves oil microcapsules.

Sample	k_0	Final absorbance (620 nm)
HSM	0.31^a	1.79^a
ASM	0.32^a	1.82^a
HCM	0.46^b	1.46^b
ACM	0.55^b	1.73^a

HSM: Hass avocado safflower oil extract microcapsules; ASM: creole avocado safflower oil extract microcapsules; HCM: Hass avocado corn oil extract microcapsules; ACM: creole avocado corn oil extract microcapsules. ^{a,b} Different letters on the same column shows significant difference ($p < 0.05$)

In Fig. 2 is possible to observe that the powders obtained from the safflower oily extracts showed a lower k_0 value in comparison with the microencapsulate corn oil extracts ($p < 0.05$), as k_0 was calculated as the rate at which the powder was dispersed, this low value showed that microcapsules of safflower oil extracts dispersed slower than the microcapsules of corn oil extracts. At the end of the dissolution time (120 min) microcapsules of safflower extracts had lower values of absorbance at 620 nm than the microcapsules of corn oil extracts finding only significant differences ($p < 0.05$) between HCM with the other samples. These results are related to the lowest encapsulation efficiency of the microencapsulation of corn oily extracts and with the high hygroscopicity value (Kang *et al*., 2019; Saifullah *et al*., 2016). The spray dried corn oil extract had more surface oil than the safflower oil extract and this caused few interactions between the hydrophilic molecules on the surface with the water molecules in the environmental air.

3.2.6 Morphometric analysis

The morphometric characteristics of the powders were obtained by photonic microscopy and processed by digital image analysis and Scanning Electron Microscopy (SEM). In Table 7 the results obtained for the morphometric parameters analyzed by the ExpertShape software are presented. The high values in perimeter and area showed that the particles formed aggregates. The largest perimeter (275.07 μm) and area (4460.54 μm^2) were obtained for the HCM sample, and the smallest perimeter (43.75 μm) and area (177.42 μm^2) were obtained for the HSM materials. HCM perimeter and area were 6-fold and 20-fold larger than powders obtained by HSM. The Feret diameter is the distance between parallel tangents on opposite sides to the particle edge, the Feret minimum diameter is the particle width and the Feret maximum diameter is the particle length. HCM results for these parameters (70.96 μm, 54.83 μm and 83.66 μm respectively) showed that HCM formed the largest particles. In addition, from values of Feret minimum and maximum, it was observed that the particles were not circular which was confirmed by the values of the ellipse ratio.

Table 7. Morphometric parameter of the microencapsulated avocado leaves oily extracts.

Measure	HSM	ASM	HCM	ACM
Perimeter (μm)	43.75	72.84	275.07	48.68
Area (μm2)	177.42	532.55	4460.54	203.45
Feret diameter (μm)	13.68	19.95	70.96	15.14
Feret mínimum diameter (μm)	11.1	15.93	54.83	12.29
Feret maximun diameter (μm)	15.71	23.22	83.66	17.35
Ellipse ratio	1.25	1.52	3.04	1.5
Elongation factor	0.6	0.65	0.75	0.63
Compactness shape factor	1.26	1.24	1.14	1.25

HSM: Hass avocado safflower oil extract microcapsules; ASM: creole avocado safflower oil extract microcapsules; HCM: Hass avocado corn oil extract microcapsules; ACM: creole avocado corn oil extract microcapsules.

When the value of this parameter is equal to 1, the particle is a circle, higher values of ellipse ratio indicates that the particles are more elongated. The obtained values are in the range of 1.25-3.04 which was confirmed through the elongation factor. The highest value of this parameter (0.75) corresponded to HCM. Compactness factor showed the degree to which a shape is compact; HCM was the particle with less compactness value (1.14). It was not possible to observe individual particles of the agglomerates by photonic microscopy due to the compactness; SEM technique was used to observe the morphology of microparticles. Through this technique it was possible to confirm that the particles arrangement was agglomerates as can be seen on Fig. 3. However, it was not possible to appreciate isolated individual microparticles. The observed morphology is similar to the morphology obtained by Böger *et al*. (2018) who use GA and MD as wall materials, the particles have a continuous wall and fusion of the particles was observed. The structure also has similarities with the obtained by Fuentes-Ortega *et al*. (2017), these authors describe that the microparticles were shriveled and concave surface and different sizes, this kind of particles are the characteristics ones of spray drying process.

3.3 Pigment quantification and antioxidant activity

Chlorophylls and carotenoids are the most ubiquitous pigments in the plants and possess high antioxidant activity (Wang *et al*., 2010). Table 8 shows the pigments quantification of avocado leaves extracts with corn and safflower oil.

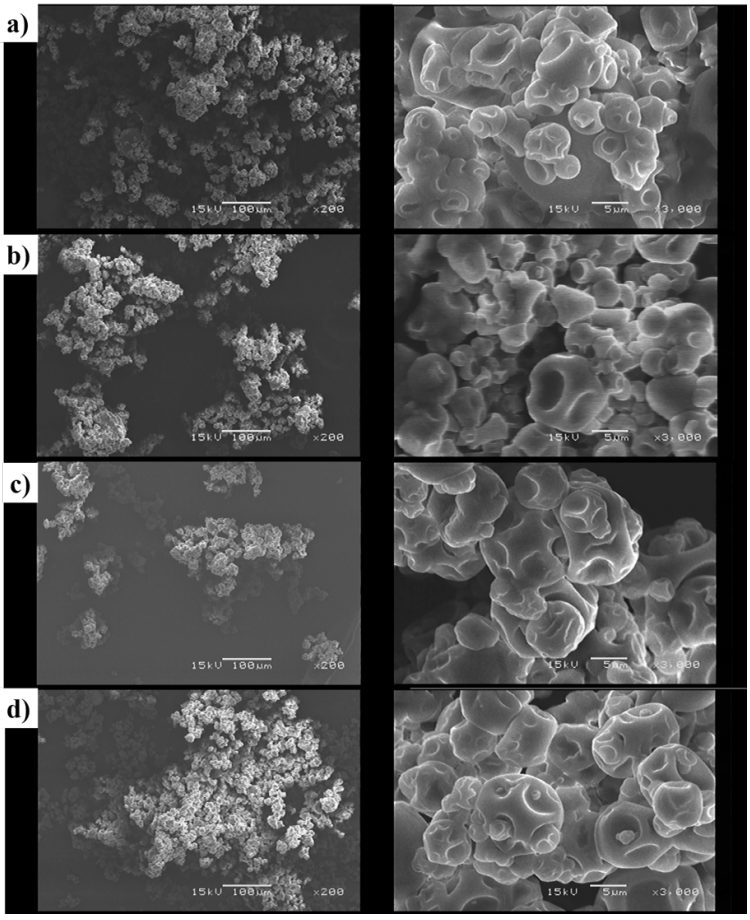

Fig. 3. SEM images at 200x and 3000x from microencapsulated avocado leaves oily extracts a) HSM: Hass avocado safflower oil extract microcapsules, b) ASM: creole avocado safflower oil extract microcapsules, c) HCM: Hass avocado corn oil microcapsules, d) ACM: creole avocado corn oil microcapsules.

Table 8. Pigment quantification of oily avocado leaves extracts.

Sample	Carotenoids (μg/g dry leaves)	Chlorophyll a (μg/g dry leaves)	Chlorophyll b (μg/g dry leaves)
HS	259.39 ± 4.12^{a}	441.29 ± 4.18^{a}	35.98 ± 6.94^{a}
AS	316.07 ± 3.97^{b}	465.98 ± 10.00^{b}	63.17 ± 6.15^{b}
HC	290.49 ± 19.31^{a}	453.73 ± 16.49^{a}	52.98 ± 3.79^{a}
AC	375.62 ± 1.88^{a}	530.58 ± 7.46^{c}	99.22 ± 6.47^{c}

HS: Hass avocado safflower oil extract; AS: creole avocado safflower oil extract; HC: Hass avocado corn oil extract; AC: creole avocado corn oil extract.

^{a,b,c} Different letters on the same column shows significant difference ($p < 0.05$)

In this table, it is possible to observe that there are differences on the pigments quantification according with the variety of avocado leaves and the vegetable oil used for the extraction. Creole avocado leaves (A) showed the higher pigment content than *Hass* avocado leaves (H) independently to the oil (corn or safflower) used for their extraction. Carotenoids content in A was approximately 20% higher than in H. Also, Creole leaves had more chlorophylls content than those of *Hass* avocado, approximately 10 and 90% more chlorophylls a and b respectively. The pigment content in the leaves could be different due to the strains and cultivars of origin (Wang *et al*., 2010). It has been demonstrated that the concentration of pigments between the sun and shade leaves of trees differ considerably (Lichtenthaler *et al*., 2007) .

Also, the leaves age affect their pigment content (Brahmi *et al*., 2013). Guadarrama-Lezama *et al*. (2012) demonstrated that the type of fatty acids chains in vegetable oils, have a direct influence on the carotenoid extraction. Corn oil can extract more pigments due to the presence of more saturated fatty acids as compared with safflower oil. The identification and quantification of carotenoids and chlorophylls of avocado leaves have not yet been reported, but it is known that avocado had carotenoids such as zeaxanthin, lutein, α- and β-carotene, neoxanthin and violaxanthin (Ashton *et al*., 2006; Wang *et al*., 2010), there are reports of pigment quantification in other avocado by-products such as skin from which higher pigment contents than pulp or seed were obtained.

Wang *et al*. (2010) reported 8.9 - 17.7 μg/g fresh weight basis and Ashton *et al*. (2006) reported 50 μg/g fresh weight basis. In comparison with these results, the pigment quantification in avocado leaves in the present work (Table 8) was 4-fold higher (259 - 375 μg/g of dry leaves).

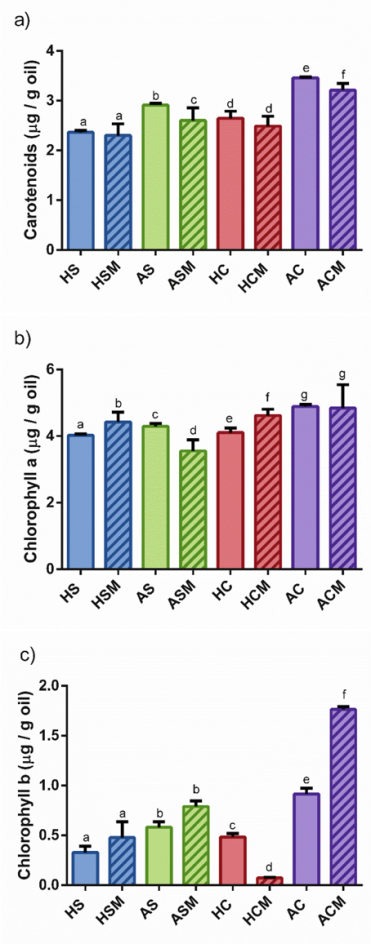

Fig. 4. Pigment content a) carotenoids, b) chlorophyll a and c) chlorophyll b on oily extracts and microencapsulated oily extracts. HS: Hass avocado safflower oil extract; HSM: Hass avocado safflower oil extract microcapsules; AS: creole avocado safflower oil extract; ASM: creole avocado safflower oil extract microcapsules; HC: Hass avocado corn oil extract; HCM: Hass avocado corn oil microcapsules; AC: creole avocado corn oil extract; ACM: creole avocado corn oil microcapsules.

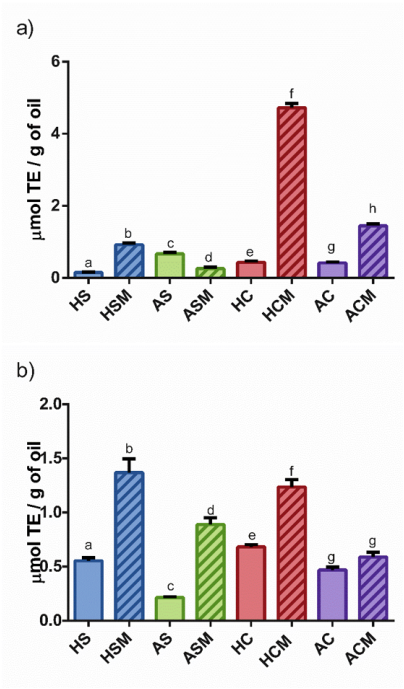

Fig. 5. Antioxidant activity by a) ABTS method and b) FRAP method of microencapsulated avocado leaves oily extracts. HS: Hass avocado safflower oil extract; HSM: Hass avocado safflower oil extract microcapsules; AS: creole avocado safflower oil extract; ASM: creole avocado safflower oil extract microcapsules; HC: Hass avocado corn oil extract; HCM: Hass avocado corn oil microcapsules; AC: creole avocado corn oil extract; ACM: creole avocado corn oil microcapsules.

The chlorophyll a content in avocado leaves was 441 - 530 μg/g of dry leaves and chlorophyll b 35 - 99 μg/g of dry leaves, these results are higher than those obtained by Ashton *et al*. (2006) who reported 70 μg/g fresh weight basis and 30 μg/g fresh weight basis for chlorophyll a and b respectively. The results obtained for pigments in avocado leaves are consistent with the fact that leaves are the plant organs that carry out the photosynthesis and chlorophylls and carotenoids are photosynthetic pigments (Lichtenthaler *et al*., 2007).

To know the vegetable oil recovery efficiency of pigments, a control extraction using hexane as dissolvent was carried out. Using hexane as dissolvent the pigment content was 221.82 ± 2.91 μg carotenoids/ g dry leaves, 318.49 ± 9.88 μg chlorophyll a/ g dry leaves and 36.55 ± 6.54 μg chlorophyll b / g dry leaves for *Hass* variety and 585.26 ± 6.18 μg carotenoids/ g dry leaves, 873.21± 2.41 μg chlorophyll a / g dry leaves and 70.51 ± 4.00 μg chlorophyll b/ g dry leaves for creole variety. As it can be seen when we compare the hexane extraction with vegetable oil extraction of pigments from H, vegetable oils showed higher amount of pigment. However, A extract did not show this behavior due to hexane showed higher values than those obtained in vegetable oils extracts.

In Fig. 4, it is possible to appreciate the effect of spray drying on the pigment quantification, spray dried conditions decreased the content of carotenoids of the avocado leaves oily extracts being significantly different ($p < 0.05$) for A extracts. It has been reported that carotenoids and chlorophylls are pigments unstable and sensitive to heat conditions, however Roshanak *et al*. (2016) demonstrate that the pigment quantification can increase after drying.

In Table 9 the antioxidant activity of avocado leaves oily extracts is shown and the effect of the microencapsulation process is shown on Fig. 5. In general, the methods to measure the antioxidant activity are classified in two groups, according with the mechanism of free radical stabilization. In the present work the antioxidant activity was measured by FRAP method which is a single electron transfer method and TEAC method that quantifies antioxidant activity via electro donation or by radical quenching by hydrogen atom transfer (Shahidi and Zhong, 2015).

The antioxidant activity measured by FRAP method in the oil extracts was significantly different ($p < 0.05$) between the varieties of avocado or the vegetable oil used for the extraction. *Hass* avocado leaves extracts showed almost 2-fold higher values of antioxidant activity that creole avocado leaves extracts. Corn extracts, showed more antioxidant activity for the two studied leaves in comparison to safflower oil extracts.

Table 9. Antioxidant activity of avocado leaves oily extracts.

Sample	FRAP (μmol TE/ g of oil)	$ABTS^{\cdot+}$ (μmol TE/ g of oil)
HS	0.55 ± 0.03^{a}	0.15 ± 0.02^{a}
AS	0.21 ± 0.01^{b}	0.67 ± 0.04^{b}
HC	0.68 ± 0.02^{c}	0.43 ± 0.04^{c}
AC	0.47 ± 0.03^{d}	0.42 ± 0.03^{c}

HS: Hass avocado safflower oil extract; AS: creole avocado safflower oil extract; HC: Hass avocado corn oil extract; AC: creole avocado corn oil extract. ^{a,b,c,d} Different letters on the same column shows significant difference ($p < 0.05$).

The results of the $ABTS^{\cdot+}$ assay for the *Hass* avocado leaves extracts showed the same behavior in comparison with the results obtained by using the FRAP method being significantly different ($p < 0.05$) the safflower extract versus the corn extract. However, the Creole avocado leaves extract with safflower showed higher antioxidant activity by the $ABTS^{\cdot+}$ assay.

Guadarrama-Lezama *et al*. (2012) demonstrated that the sort of carotenoid that each fatty acid chain can extract are different, and for this reason the antioxidant activity of each extract could be different since each carotenoid has a different activity. Safflower oil has less saturated fatty acids (7.4%) than the corn oil (12%) which caused lower carotenoids extraction with antioxidant activity.

Results obtained of the antioxidant activity were much lower than those reported by Liu *et al*. (2018) when studying different species of crabapple cultivars (between 100-277 mmol TE / g) these authors attributed the antioxidant activity to the phenolic compounds. Therefore, the differences between our results and those reported previously for different sort of leaves, might be due to the polarity of the solvent used to extract the bioactive compounds of the leaves. Since the solvent used in this work has a non-polar character, it was not possible the extraction of phenolic compounds that confer high antioxidant activity.

Conclusions

Chlorophylls and carotenoids from avocado leaves were extracted using vegetable oils as solvents; this could be possible due to the lipophilic characteristics of the pigments. The microencapsulation of these compounds using spray drying process resulted as a good alternative to preserve the extracted pigments. The pigments concentrations remain relatively constant before and after spray drying process. Spray drying microencapsulation showed low encapsulation efficiency, the presence of superficial oil improve the agglomerate formation limitating powders flowability and dissolution capacity. Besides the fact that the corn oil extract showed higher pigments contents for both leaves, the microencapsulates showed less dissolution capacity and higher hygroscopicity values caused by the dissolution of wall materials. Further analyses are necessary to known the extracts biocompounds profile.

Acknowledgements

The first author is grateful to CONACYT for the PhD scolarship granted (254415), also the authors wish to acknowledge the financial support provided by Secretaría de Investigación y Posgrado - Instituto Politécnico Nacional (SIP - IPN) through the projects numbers 20170459, 20170428, 20180694, 20181309. The authors appreciate and thank the support given by Dr. Edgar Oliver López Villegas at IPN -ENCB Central Microscopy, for the SEM images obtained.

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