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Microencapsulation of *Moringa Oleifera* leaf powder by gelatin-polysaccharide complex

Microencapsulación de polvo de hoja de *Moringa oleifera* por complejo de gelatina-polisacárido

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Abstract

Moringa Oleifera leaves contain a large number of essential nutrients, but their organoleptic characteristics could limit their inclusion as a dietary supplement. This paper proposed a microencapsulation method based on complex coacervation for Moringa Oleifera leaf powder (MLP) by using gelatin combined with a polyanion (Xanthan Gum, X, or Carboxymethyl Cellulose, CMC) , protecting MLP to ameliorate its properties. During the study, the gelatin/polymer ratio and pH were adjusted for each pair Gelatin:Polysaccharide (G:Ps). In the absence of MLP, complex formation percentages of 98%, G:X, and 100%, G:CMC, were obtained. A micrographic analysis of the microencapsulated MLP for each G:Ps pair served to select Xanthan Gum (X) for the definitive microcapsule G:X, due to its better adhesion on MLP surface; a final 73% MLP coacervation percentage was obtained. Microcapsules G:X exhibited 32% in crude protein content, 6% ash, and a brown-green color, therefore revealing the potential of microencapsulated MLP as a nutritional supplement.

Keywords: Moringa Oleifera; Micro-capsule; Interpolymer complexes; Complex coacervation.

Resumen

Las hojas de Moringa Oleifera contienen una gran cantidad de nutrientes esenciales, pero sus características organolépticas podrían limitar su inclusión como suplemento dietético. Este documento propone un método de microencapsulación basado en la coacervación compleja para el polvo de hoja de Moringa Oleifera (MLP por sus ciclas en inglés Moringa Leaf Powder) mediante el uso de gelatina combinada con un polianión (goma xantana, X o carboximetilcelulosa, CMC), protegiendo al MLP para mejorar sus propiedades. Durante el estudio, la relación gelatina / polímero y el pH se ajustaron para cada par Gelatina: Polisacárido (G: Ps). En ausencia de MLP, se obtuvieron porcentajes de formación compleja de 98%, para el dúo G: X y 100%, en el par G: CMC. Un análisis micrográfico del MLP microencapsulada para cada par G: Ps sirvió para seleccionar la goma xantana (X) para la microcápsulación definitiva G: X, debido a su mejor adhesión en la superficie del MLP. Se obtuvo un porcentaje final de coacervación del MLP del 73%. Las microcápsulas G: X exhibieron 32% en contenido de proteína cruda, 6% de cenizas y un color verde-marrón, revelando así el potencial de MLP microencapsulado como suplemento nutricional.

Palabras claves: Moringa Oleifera; Microcápsula; Complejos interpolímeros; Coacervación compleja.

1 Introdución

In developing countries, problems associated with overpopulation, the limited income, and the high cost of animal source food, have motivated the search for new plant alternatives to address the need for nutrient intake. *Moringa Oleifera* contains a high nutritional value and may contribute to population health, especially the leaves, which contain proteins, minerals, vitamins, mainly A, C and E, carotene, and antioxidant compounds (Gopalakrishnan et al., 2016). In recent years it has been incorporated into food products to increase its nutritional value. Some applications of food fortification have been the manufacture of soups, weaning foods, amala (a dough made of yam and flour of banana), herbal biscuits, bread, cake, and yogurt (Oyeyinka et al.,, 2016 and Bourekoua et al., 2018).
Despite its great benefits, studies indicate rejection or low acceptance of some products fortified with Moringa leaf, mainly by children, due to the color and slightly bitter taste that leaves this plant when ingested (Nambair et al., 2008, Bourekoua et al., 2018).
Then, several techniques have been developed to address this problem. Of them, the encapsulation of powdered substrates, also called micro-encapsulation, is one of the most used methods. Micro-capsules provide support, protection, or coating qualities of active ingredients that require their properties to be stored until they are deposited in the right place and time. Some of the properties and agents that have improved their functionality through these methods have been the flavors, aromas, textures, enzymes, drugs, among others (Munmaya Mishra, 2016).

Complex coacervation is an encapsulation method based on the ability of water-soluble polymers and opposite charges to interact with each other and form a combination polymer phase capable of segregating in the aqueous medium. Coacervates are generally produced from proteins and polysaccharides. They can have a wide range of functions in various food products and, in particular, can be applied in the encapsulation of solid food substrates (Sengupta et al., 2017). For example, microencapsulation of broccoli particles has been proposed to reduce its characteristic aroma (Sánchez et al., 2016).

The aim of this paper is to propose a microencapsulation method based on complex coacervation for *Moringa Oleifera* leaf powder (MLP) by using gelatin combined with a polyanion (Xanthan Gum, X, or Carboxymethyl Cellulose, CMC). We begin by determining the primary parameters in the polymer complex formation (Section 3.1). These results are used to obtain the MLP microcapsule (Section 3.2), which is then characterized in Section 3.3.

2 Materials and methods

2.1 Materials

UTM Food Science and Technology Laboratory Research Group provided *Moringa Oleifera* leaf powder (MLP), prepared with Moringa leaves from Mariscala de Juárez, Oaxaca, Mexico, hand washed and disinfected in hypochlorite solution at 125 ppm for 15 minutes (Suslow, 2000). The leaves were dried in a Felisa oven at 50 ° C for 8 hours. They were then crushed in a Krups GX4100 mill until a fine powder was obtained, which was screened in an RO-TAP RX-29 with No. 80 mesh sieve (Dachana, et al., 2010).
For capsule formation, food brand polymers were used: Gelatin (G) Gelita brand TypeA-TypeB mix, with Bloom 280-310 (corresponding to a molecular weight between 77-93 KDa), Xanthan Gum (X) distributed by Cedrosa with molecular weight greater than 1000 kDa and the DEIMAN brand Carboxy-Methyl-Cellulose (CMC) with molecular weight between 90 and 700 kDa. In the pH control, citric acid and/or sodium hydroxide Sigma brand was used. Coacervation was performed in an aqueous medium with water purified by reverse osmosis. For the protein determination, sulfuric acids (Meyer brand), hydrochloric and boric acids (Baker brand), copper pentahydrate and, anhydrous potassium sulfates (Meyer brand) and the Wesslow indicator (methyl red and methylene blue, Meyer brand) were used.

2.2 Complex Coacervation Method

Complex coacervation in aqueous solution is obtained by mixing a negatively charged polymer solution (Xanthan gum, X or Carboxymethyl Cellulose, CMC), added over a protein solution (gelatin, G) with pH values below 5 to guarantee a charge positive and achieve electrostatic interaction. Parameters studied that impact the coacervation of MLP were: the pH of the gelatin and polyanion mixture solution, the mass ratio between the polymers of the mixture (G: P), the type of polyanion used (X or CMC) and the concentration of polymer solutions.
In a beaker at room temperature T = 22 ° C ± 1, a given volume of the gelatin solution from 0.5 to 1%, prepared with a buffer solution of citric acid and NaOH, was added to the required pH value. Under magnetic stirring, a known volume of CMC solution of 0.25 to 0.5% is added drop wise to the beaker. The amounts of the mixed solutions depended on the desired ratio Gelatin: Polyanion (G: P). For all tests, total mass of polymer remained constant at 0.27 grams. Immediately after completion of the mixing, the pH of the solution was verified and adjusted to the required pH by adding a 0.5 M citric acid solution if necessary. The system was allowed to stir for

15 minutes to admit of coacervate formation, which was filtered off. The precipitate was washed with water, dried in an oven at 60 ° C to constant weight, and used to determine the yield of coacervate (%). The supernatant filtrate was reserved for transmittance measurements.

Initially, for each polyanion, one-dimensional pH scans were performed at a ratio Gelatin:Polyanion constant, with a solution of 1% gelatin (w / v) in water, 0.2% xanthan gum solution (w / v) and CMC at 0.5% (w / v), in the corresponding cases. Subsequently, keeping the pH constant at the maximum coacervation percentage (pHmax) value, the percentage of gelatin in the polymer mixture was varied. The interaction between gelatin and polyanion was considered at its maximum in terms of coacervate yield (%) and transmittance measurements.

Encapsulation of MLP was performed at the identified pH and G: P ratio. The powder was added to the gelatin solution in a proportion of 10 grams of MLP for each gram of total polymers.

Influence of the polymer solution concentration was evaluated under the optical microscope by relative level observations of coacervate adhesion on the surface of the solid. Concentration levels in each solution were varied from a low to a high value, being 0.5-1% for gelatin, 0.1-0.2% for Xanthan and 0.25-0.5% for CMC. The encapsulated MLP was dried and ground to characterize the color, protein content, and ash under similar conditions compared to the original MLP.

2.3 Characterization Methods

Coacervate yield (%)

The coacervate yield (%) obtained by mixing the CMC or Xanthan and the gelatin, in different proportions, were determined by the gravimetric method. The ratio (mass of the recovered coacervates) / (total polymer mass added at the beginning) represents the coacervation percentage. The behavior observed by the coacervation ratio was corroborated by transmittance measurements at a wavelength of 500 nm using a UV-visible spectrophotometer.

Crude protein determination

The determination of the crude protein content (Method 954.01 of the AOAC, 1997) was performed in triplicate by digestion with concentrated H2SO4 (2.5 mL) of 0.1 g of the powder sample, in the presence of catalysts (40 mg of CuSO4·5H2O, 1.3 g of K2SO4). The subsequent distillation process was performed in the presence of 50% NaOH (10 mL), and the distillate was received in a solution of 4% H3BO3 (5 mL) with Wesslow indicator. The receptor solution was titrated with a 0.1 N HCl solution. Calculations for obtaining% total nitrogen and crude protein were performed according to equations 1 and 2.

$$TN(\%) = \frac{(V_2 - V_1) \times N}{W} \times 14\ x\ 100 \quad (1)$$

$$CP(\%) = TN(\%) \times CfN \quad (2)$$

where V2 is the volume of HCl solution required for the test sample (ml), V1 is the volume of HCl solution required in the blank test (ml), W is the test sample mass (g), N is the HCl solution normality, CP (%) is the percentage of crude protein, TN (%) is the total percentage of nitrogen and CfN is the nitrogen conversion factor used as 6.2

Ash Determination

For the determination of the percentage of ashes (Method 923.03 of the AOAC, 1997), 3 g of powdered sample was weighed in a constant weight crucible. Sampling burned slowly on an electric grill until combustion was completed. Subsequently, it was calcined in a muffle (Felisa) at 550 ± 10 ° C, until the ashes acquired a white color. Finally, the crucible was frightened in an airtight desiccator and weighed. This determination was made in triplicate and calculated by equation 3.

$$Ashes\ (\%) = \frac{(W_3 - W_2)}{W_1} \times 100 \quad (3)$$

where W_3 is the crucible with the ashes mass (g), W_2 the empty crucible mass (g), and W_1 is the sample mass (g).

Color determination

The color parameter was quantified under the CIE system. The CIELAB and CIELCH coordinate measurements of MLP alone (without encapsulation) and microencapsulated were analyzed with the CR-5 colorimeter (Konica Minolta ®), under the standard conditions of specular reflectance excluded (SCE) and daylight illuminant (CIE-D65).

The CIELAB and CIELCH color space allow the color to be measured by reading the rectangular coordinates (L *, a *, b *) considered dimensionless magnitudes and cylindrical coordinates (L *, h *, C *) respectively. The names and values of the coordinates are: L * = luminosity or clarity (values between 0-black and 100-white), a * (towards the red positive value (+), towards the green negative value (-)), b * (towards yellow positive value (+), towards blue negative value (-)), C * = chroma (values between 0 to 150 for achromatic stimuli) and h * = tone (varies between 0 and 360º and for achromatic stimuli (a * = b * = 0) is an indefinite magnitude).

Chroma (C *), tone (h *) and color difference (ΔE) are determined by the following equations (Mathias-Rettig et al., 2014)

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (4)$$

$$H^* = arctg\left(\frac{b^*}{a^*}\right) \quad (5)$$

$$\Delta E = (L^{*2} + a^{*2} + b^{*2})^{1/2} \quad (6)$$

3 Results and discussion

3.1 Gelatin-CMC and gelatin-Xanthan complex formation

The variation of pH modifies the charge of the ionizable groups that make up the biopolymers. To each protein-polysaccharide pair, there corresponds a composition ratio and a pH value for which the charges of each macromolecule are opposite and equivalent. This pH value is known as the electrical equivalence pH (EEP) and corresponds to the maximum performance of the formation of the electrostatic complex, as well as, the higher amount of the coacervated phase (Burgess et al., 1984).

For the B Gelatin and CMC coacervates, the proposed pH values range between 3 and 4.5 for percentages of gelatin in the biopolymer mixture, between 50 and 90% (Duhoranimana et al., 2017). Devi and Maji, 2011 found in the encapsulation of Neem oil, a high coacervation between type A gelatin and CMC for a pH of 3.5 and 70% gelatin in the polymer mixture.

In the present work, the determination of the conditions to achieve the greatest coacervation value between the commercial gelatin (G) and the (CMC) were obtained by executing one-dimensional sweeps of these two parameters. First, a pH scan was performed for a constant ratio of Gelatin and CMC from 4 to 1, concerning the total polymer mixture used in coacervation, which is 75% Gelatin (1% solution) and 25% CMC (0.5% solution).

The coacervation percentage (bars on Figure 1) increases as the pH do so until reaching a maximum coacervation point at a pH of 3.6, once achieved this maximum point, the mass obtained from the coacervated complex decreases as the pH continues to increase.

Another analytical technique for the identification of the maximum coacervation point, proposes measures of light dispersion of the mixing solution during pH titration, in this case, the intensity of the scattered light passes through a maximum indicating a mass and a maximum number of electrostatic complexes formed (Li, et al., 1994). As our application focuses on a maximum amount of precipitated complex, to achieve a solid matrix coating and avoid the influence of kinetic type parameters, it was preferred to measure the transmittance of the separated solution after filtration of the precipitated complex, for each pH value. In Figure 1, the transmittance measurement is represented by a solid line, which shows a high value for the maximum precipitation pH, which gives an indication of reduced polymer concentration in the solution. Outside this point of maximum precipitation, complexes remain soluble due to insufficient charge neutralization (Weinbreck et al., 2003), resulting in a decrease in solution transmittance.

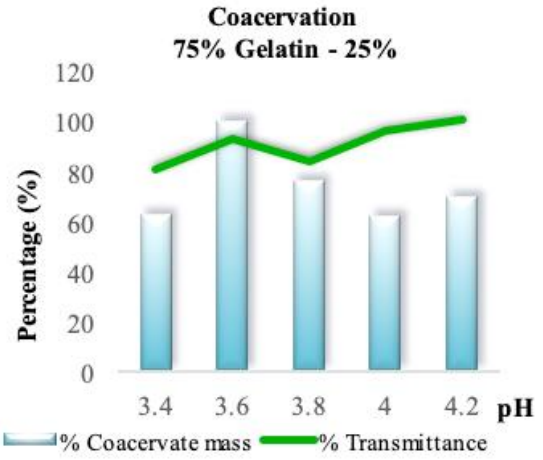


Fig. 1. Percentage of coacervation of the precipitated complex (bars) and transmittance (at 500 nm) of supernatant solution (solid line), using 75% Gelatin (1% solution) and 25%CMC (0.5% solution).

For the Gelatin-Xanthan mixture, fewer studies on coacervate formation a reported in the literature. Wang et al. (2016), whose work aimed at obtaining and characterizing hydrogels, collaterally report the electrostatic complex precipitation, which appears for pH values below 4.5, in a polymeric mixture with 83% of gelatin. Another study, aimed at the formation of Gelatin-Xanthan complexes by electrosynthesis, mentions the formation of a G-X complex at a 1: 1 ratio for a pH of 2.5 (Lii, et al., 2002). However, no composition and pH conditions are reported for high performance in the G: X complex formation. In this work, the coacervation study began with a composition of 50% gelatin and 50% (Xanthan), mixing solutions of 1% and (0.2%), respectively. At this fixed composition, coacervations are carried out for pH value from a value of 3.4 to 4.2. In Figure 2, transmittance percentages in the supernatant solutions, after filtering the precipitated coacervates, are observed comparatively for the two Gelatin-Polysaccharide (G: P) pairs studied. In both systems, the maximum pH value is 3.6; the behavior of both systems differs for the values of the transmission at a higher pH. The transmittance values for G: X (first band flat) remain low, while for the G: CMC solutions increase again when they move away from the optimum point. This behavior responds to the difference in the transmittance of individual polyanion solutions, which are 52% for the Xanthan solution and 100% for the CMC solution. If these values are compared with those of the supernatant solutions for pH 4.2, (equal to 55% for the mixture G: X and 100% for G: CMC), it can be thought that these solutions contain a large polysaccharides portion non-precipitated.

All these results allow us to propose transmittance measurements of the supernatant solutions to the

precipitated complex, where a local increase can show the point of maximum coacervation, regardless of the transmittance value of the mixed individual polymer solutions.

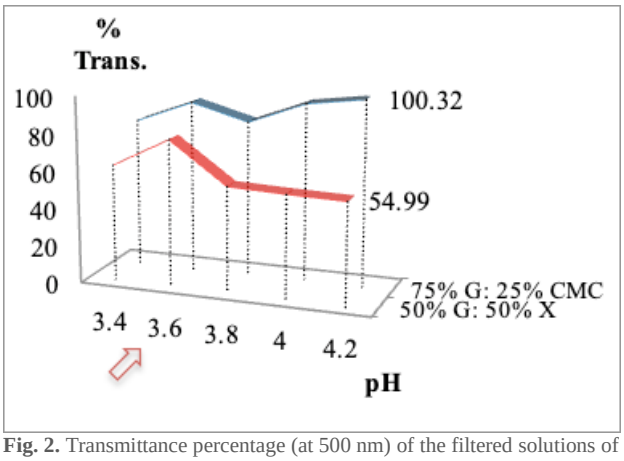


Fig. 2. Transmittance percentage (at 500 nm) of the filtered solutions of the coacervate, for the 50:50 ratio gelatin-xanthan mixtures (red band in the foreground) and 75:25 ratio of Gelatin-CMC (band blue in background).

In the second step, for the constant pH value of 3.6, a G: P ratio was found for which a higher percentage of coacervation is observed for each studied polysaccharide. Percentage values are presented in bar form in Figure 3, accompanied by the transmittance percentage values of the separate solutions, represented in the superimposed continuous line. On the left (Figure 3 a), the G: CMC system achieves the maximum coacervation ratio in a 75% polymer gelatin mixture, confirming the starting composition. While for the coacervate G: X, the higher recovery composition, is adjusted at 65% gelatin. In both cases, maxima are obtained for gelatin-rich mixtures; however, as expected in an equivalent molar contribution, for CMC with lower molecular weight than Xanthan gum, less polysaccharide percentage is required to provide a similar number of moles (25% for CMC, less than 35% xanthan gum).

At these optimized points, values close to or equal to 100% are reached, practically integrating the total of polymers in solution into intermolecular complexes of sufficient size to precipitate. This fact is corroborated with transmittance values of the supernatant solution close to 100%, which shows the absence of non-precipitated complexes. On the one hand, in the case of the G: CMC mixture (where pure polymer solutions have 100% transmittance at that wavelength), the observation of transmittance values less than 100%, before and after the optimum point, indicates the presence of complexes in colloidal size solutions that fail to precipitate and are suspended in the solution.

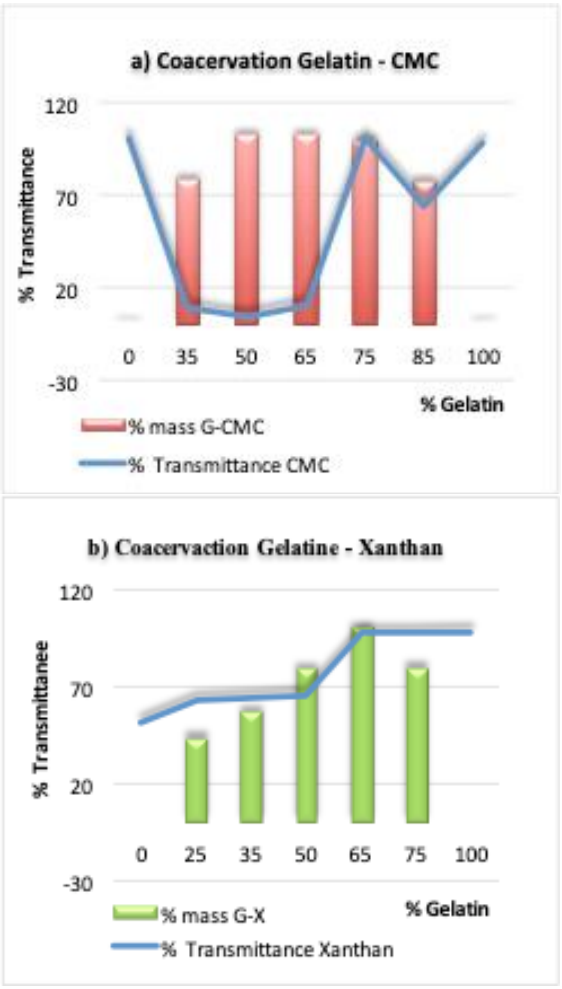


Fig. 3. Coacervation and transmittance percentages obtained by varying the percentage of gelatin in the polymer mixture for the systems a.) Gelatin-CMC and b.) Gelatin-Xanthan. At pH 3.6.

On the other hand, the G: X mixture showed, after the maximum point, transmittance values close to 100% that indicate the absence of suspended complexes, or the xanthan gum without precipitating; in this case, the non-precipitated mass would only be composed of gelatin in solution. These observations are in agreement with the mechanisms of formation and stabilization of complexes reported in the different zones of pH and composition by (Wang et al., 2016 and Duhoranimana et al., 2017).

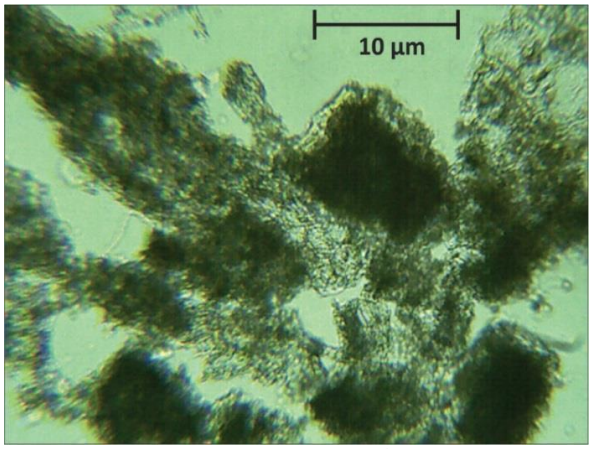
3.2 Moringa micro-encapsulation

Once the conditions of pH and polymer composition for maximum complex formation have been determined, it is now important to ensure that the complex formed is deposited on the solid surface of the powder and forms high-coated capsules. At this stage, the influence of the mixed solutions concentration on the efficiency of this deposition is evaluated since, as predicted by Von Weimarn's Precipitation Theory (Buchner and Kalff,

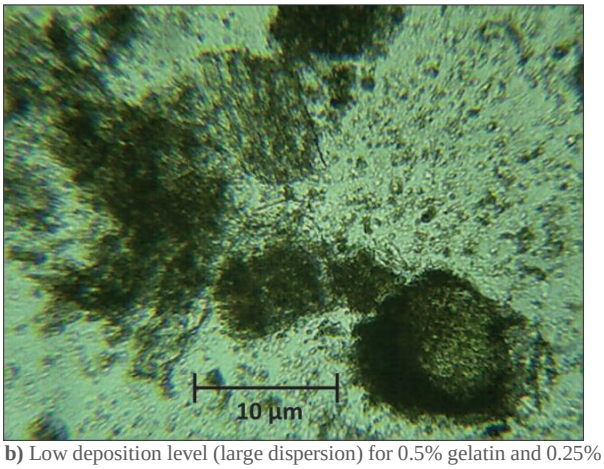
1920), the concentration factor can modify the effective precipitation of the complex. Coacervation tests were carried over Moringa powder, with solutions of two concentrations: equal and with half the concentration of those used in the previous section. Solutions of Gelatin at 1% and 0.5% were studied, to interact with solutions of CMC at 0.5% and 0.25% and Xanthan at 0.2% and 0.1%. Solutions of Gelatin at 1% and 0.5% were studied, to interact with solutions of CMC at 0.5% and 0.25% and Xanthan at 0.2% and 0.1%. The morphology and level of deposition of the coacervate formed on the powder were observed by microscopy, classifying the degree of effective deposition into three levels. Figure 4-a, show examples of low deposition levels, with transparent appearance coacervates distributed mainly on the surface of the solid, and the solution does not show dispersed coacervates.

Figure 4-b shows a low adhesion level with little amount of coacervates on the surface and a large amount of polymer dispersed in the solution. Another important observation is the relative behavior of the two studied pairs. On the one hand, the G/CMC pair goes from a high coating to a low level when diluting the mixed solutions, while the G/X pair seems not to alter its high coating with said dilution. This relative stability of the G/X complex to the concentration of the mixing solutions makes it possible to select the coacervation of G/X for the micro-encapsulation of moringa in successive evaluations. The impact assessment of the concentration was completed for the G/X mixture with a two-factor factorial study at two levels (2^2), with the concentrations of the solutions (Cc.G) and (Cc.X) as variables and the coacervate deposition level on the solid surface, in response. The results of this design presented in table 1 were quantified according to the high, medium, and low levels as three (3), two (2), and one (1), respectively.

An adjustment model is obtained through the origin, between the concentration factors and the responses in Table 1. In the model, the coefficients of the independent concentration are negative, showing that the increase in some concentration can decrease the deposition, by favoring nucleation and de-favoring colloidal growth, as Von Weimarn's theory suggests (Buchner and Kalff, 1920). The model also shows the impact of the interaction factor of both concentrations (term Cc.G * Cc.X), which presents a positive coefficient of greater magnitude than the individual concentration variables. This magnitude suggests that the parallel increase of both concentrations contributes positively to the polymer complex deposition.



a) High deposition level (little dispersion) for 1% gelatin and 0.2% Xanthan, solutions



b) Low deposition level (large dispersion) for 0.5% gelatin and 0.25% CMC, solutions

Fig. 4. Micrografie and coacervated deposition level on Moringa leaf powder, for mixing Gelatin-Xanthan and Gelatin-CMC polymeric solutions at various concentrations. a) high and b) low deposition.

This micrographic study also allows contrasting the elongated morphology of the G-X complexes (Figures 4-a) compared to the granular complexes of G-CMC (Figure 4-b). Similarly, in the work of Lanveuville et al., 2005, it is proposed that xanthan gum acts as the support for the formation of primary complexes, and increasing their molecular weight, larger interpolymeric complexes are produced. Other studies have reported a similar influence: for cationic polymers in interaction with opposite micelles, the molecular weight increase of the polyelectrolyte results in the formation of larger primary complexes with higher aggregation in intermolecular complexes that precipitate (Turgeon et al., 2007), as well as, an increase in the coacervation zone and coacervated volume fraction (Wang et al., 2000).

Table 1. Matrix of test design to assess the polymer solution concentrations effect on coacervation behavior.

Deposition level	HIGH (3)	LOW (2)	LOW (1)	HIGH (3)
Gelatin concentration	0.5%	1%	0.5%	1%
Poli-anion concentration	0.1%	0.1%	0.2%	0.2%

3.3 Micro-encapsulated Moringa characterization.

The characterization tests of the Moringa microcapsules were performed in duplicate, on the samples obtained by depositing complex with 65% gelatin and 35% xanthan gum for a pH of the solution at 3.6. After filtering, washing, and drying, the amount of microencapsulated Moringa recovered was 73% ± 1. The missing mass for 100% represents the components that go into solution during the coacervation process, such as possible mineral and organic Moringa compounds soluble in the aqueous medium (Nweze and Nwafor, 2014). An efficiency of 73% represents an average value comparing at the emulsified systems coacervation, where the values can vary between 16 and 90% (Zhu and Yunwei Niu, 2014). This efficiency represents a high value compared to those reported in similar matrices, such as 58% in the coacervation of broccoli powder (Sánchez et al., 2016).

The physicochemical analyses were oriented to three essential characteristics for moringa as a food product or supplement. The crude protein percentage and the ashes, which guarantee the moringa leaves nutritional contribution and the color, which is part of the sensory parameters that could generate rejection levels in the food presentation.

The crude protein content of the unencapsulated Moringa leaf powder is 28.21%, consistent with reported values for moringa grown in Mexico, ranging between 21 and 29% (Melo et al., 2013, Guzmán-Maldonado et al., 2015), The apparent increase in protein content makes it clear that the lost mass is mainly composed of non-protein compounds, thus concentrating the amount of protein in the microcapsules. On the other hand, the ash content obtained, which implies the percentage of minerals (Nweze and Nwafor, 2014), shows a percentage of 11.751% ± 0. 04 for dust without encapsulation and 6,212% ± 0.004 for microcapsules, showing as expected a more significant amount of loss in these components.

Based on these results, it was calculated that for 100 g of original MLP, 23.83 g and 4.53g of ashes are preserved for microencapsulated presentation, therefore, despite some moringa components waning during the process, Microencapsulated MLP represents a food supplement with a high protein level (32.64%) and an significant mineral contribution (6.21%).

Fig. 5. Visual appearance difference between *Moringa Oleifera* powder alone (A) and encapsulated (B)

The coacervation effect on the MLP color can be seen visually in Figure 5, where moringa powder (fig.5A) is characterized by a bright green color, while the micro-capsules have a brown hue; these color characteristics are detailed in table 2, with the indexes of the CIE-LAB and CIELCH methodology, especially the (a *) parameter that passes from a negative value indicative of green tones for dry moringa, towards a positive value indicating approach to red tones for the micro-encapsulated MLP. At the same time, the parameter (b *) indicates its chromatic permanence within the yellow tones with a slight decrease.

The authors Koca et al., 2006, pointed out as general acceptance in the literature that the leading cause of green discoloration during processing vegetables is the chlorophyll conversion into pheophytins due to the influence of pH and heating. Mosquera et al., 1989 showed that during this reaction, hydrogen ions could transform chlorophylls into their corresponding pheophytins by magnesium ion replacing in the porphyrin ring. The lower the pH, the higher the dissociated hydrogen ions concentration, and the higher the rate of color change. The chlorophyll conversion into pheophytin and feoforbide results in a change from bright green to opaque yellow-olive green, which in the case of studies on broccoli, Gunawan and Barringer, 2000 considered it unacceptable, while, for the processing of olives, Mosquera et al., 1989, denote it as a quality attribute.

In agreement with these observations, the green-brown hue acquired by the micro-capsules is due to the coacervation process in an acid medium. A similar color change was found by (Foo Looi et al., 2019) in micro-capsules of MLP using methanolic extract with maltodextrin in spray drying. The authors observed that the particles take a golden color due to maltodextrin concentration and drying temperature, finding this coloration as an attribute, which added to the phytochemicals retention gives the micro-capsules a potential application in healthy foods production.

Table 2. Color measurements applying CIELAB and CIELCH models for *Moringa Oleifera* leaf powder (MLP) alone (A) and 65% Gelatin - 35% Xanthan MLP encapsulated (B).

	Sample	(A) Moringa leaf powder MLP	(B) MLP Coacervated
CIELAB color model	*Luminosity (L*)*	*50.4*	*38.06*
	Red scale (+) to green (-) (a)*	*-7.77*	*1.09*
	Yellow scale (+) to blue (-) (b)*	*29.44*	*21.91*
CIELCH color model	*Chromaticity (C*)*	*30.34*	*21.94*
	Tonality (h)*	*104.79*	*87.14*
	Color difference (ΔE)	*58.88*	*43.93*

4 Conclusions

The complex coacervation method can be applied for the *Moringa Oleifera* leaf powder micro-encapsulation, using commercial gelatin and xanthan gum as bio-polymers in a 65% -35% ratio respectively and 3.6 pH solution. This method allows reaching coacervation percentages of 73% when using a polymer / MLP ratio of 1 to 10. These micro-capsules keep the nutritional properties plant, providing 32% crude protein and 6% ashes. The green-brown color acquired by micro-capsules could reduce the change color impact experienced by foods fortified with leafs moringa powder due to their original bright green tone.

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Authors' contributions.

Lorena Barragán-Noriega performed the experimental part for the moringa leaf powder microcapsules synthesis, its characterization, and results-analysis; it also contributed to the writing of this work. Laura Márquez De Santis contributed to the organization and execution of the experimental work, results-analysis, and writing of the work. Mirna Patricia Santiago Gómez supplied the moringa leaves powder, participated in the microencapsulated MLP characterization and the writing of the work. Rosa Isela Ruiz Ruiz participated in the preparation of powder and micro-capsuled moringa as well as in their characterization. All authors approved the final versión.

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