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DOUBLE EMULSION AND COMPLEX COACERVATION IN STEVIA ENCAPSULATION

DOBLE EMULSIÓN Y COACERVACION COMPLEJA EN LA ENCAPSULACIÓN DE ESTEVIA

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ABSTRACT

Background: Stevia leaves a residual flavor at moment of being consumed, and its sweet taste remains little time, whereby, encapsulation is an option to mitigate these problems. **Objective:** Evaluate the double emulsion system followed by complex coacervation in stevia encapsulation. **Methods**: The effect of the concentration of the sweetener was determined (3.5; 5; 7.5 and 10% p/p) as well as the concentration of the wall material (2.5 and 5% p/p), on the morphology, capsules size, and encapsulation capacity. The double emulsion was prepared, the coacervate was formed, and then capsules were lyophilized. The morphology and capsule size were measured before and after lyophilization by optical microscopy. From Fourier´s infrared transformed spectrometry, encapsulation capacity was analyzed. Water activity and solubility were measured in lyophilized capsules. **Results:** Micro and nanocapsules (minimum size of 19.39 ± 0.74μm and 62.33 ± 6.65μm maximum) were obtained. Micrographs showed that the encapsulation technique used, allows obtaining dispersed stevia capsules and those of round and homogeneous morphology. The encapsulation capacity was 84.37 ± 4.04%. The minimum value of water activity was 0.49 ± 0.01 and 17.65 ± 0.91% of solubility. **Conclusions:** An increased in encapsulation capacity was obtained when the highest concentration of the wall material was used. The capsule diameter increased as the sweetener concentrations increased. The formulation to 5% (p/p) of stevia and 5% (p/p) in wall material was associated with better controlled release of the sweetener, which allows establishing subsequent applications in which the sweet taste is prolonged and the stevia bitter taste concealed.

Keywords: Stevia, natural sweetener, capsule, complex coacervation, double emulsion.

RESUMEN

Antecedentes: La estevia deja sabor residual al ser consumida, y su sabor dulce permanece poco tiempo, por lo cual, la encapsulación es una opción para mitigar estos problemas. **Objetivo**: Se evaluó el sistema doble emulsión seguido por coacervación compleja en la encapsulación de estevia. **Métodos:** Se determinó el efecto de la concentración del edulcorante (3.5; 5; 7.5 y 10% p/p) y de la concentración del material de pared (2.5 y 5% p/p), en la morfología, tamaño de cápsulas, y capacidad de encapsulación. Se elaboró la doble emulsión, se formó el coacervado, y posteriormente, las cápsulas se liofilizaron. La morfología y el tamaño de las cápsulas, se midieron antes y después de la liofilización mediante microscopia óptica. A partir de espectrometría infrarroja de transformada de Fourier se analizó capacidad de encapsulación. En las cápsulas liofilizadas se midió actividad de agua y solubilidad. **Resultados:** Se obtuvieron micro

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y nanocápsulas (tamaño mínimo de 19.39±0.74μm y máximo 62.33±6.65μm). Las micrografías indicaron que la técnica de encapsulación usada, permite obtener cápsulas de estevia dispersas y de morfología redonda y homogénea. La capacidad de encapsulación fue 84.37±4.04%. El valor mínimo de actividad de agua fue 0.49±0.01, y solubilidad de 17.65±0.91%. **Conclusiones:** Se obtuvo incremento en la capacidad de encapsulación cuando se utilizó la mayor concentración del material de pared. El diámetro de las cápsulas aumentó a medida que se incrementaron las concentraciones del edulcorante. Se concluyó que la formulación a 5% (p/p) de edulcorante y de 5% (p/p) en material de pared fue el tratamiento que mejor se asocia a una liberación controlada de estevia, lo cual permite establecer posteriores aplicaciones en las que se prolongue el sabor dulce y enmascare el sabor amargo de la estevia.

Palabras clave: Estevia, edulcorante natural, cápsula, coacervación compleja, doble emulsión.

INTRODUCTION

In the food industry the use of additives allows to increase the shelf life of products, counteract bacterial degradation, provide attractive colors to food, enhance the perception of flavors and scents, among others (1, 2). Stevia is an additive classified as a non-caloric sweetener, which has been extensively studied in countries such as Japan and the Far East (3), and it is highly consumed in in The United States and China (4). Stevia used in beverages, chewing gum, baked goods, yogurt, as toothpastes components and mouthwashes. It is used as a sweetener in liquid, powder or tablets (5). Within its composition, stevioside is the main component of stevia, which is characterized by its sweetening power, which is from 250 to 300 times sweeter than sucrose. It is known that the main disadvantage of stevioside is its bitter taste, undesirable factor for direct applications in food matrices (6).

A viable alternative in order to hide the bitter taste of stevioside, for use in foods, could be the technology called encapsulation by double emulsion followed by complex coacervation. The technique refers to the process by which after the formation of a simple emulsion containing the component to be encapsulated, a double emulsion is formed, emulsion which is formed by adding two polymeric materials generally a polysaccharide and a protein, and appropriate emulsifiers.

Subsequently, by pH change, a phase separation occurs spontaneously, and the coacervate is formed. The coacervate is formed by the electrostatic interactions between the polymers of the wall material is formed, which causes formation of an insoluble gel state called capsules, microcapsules, nanocapsules or, depending on their size. The encapsulation process by complex coacervation ends with the coacervate dehydration, which confers stability to the capsules (7). The formation of the double emulsion can be water-oil-water type ($W_1/O/W_2$) or oil-water-oil type ($O_1/W/O_2$) (8-11).

Through this technique functional compounds can be encapsulated (12, 13). Additionally that technique allows preparing micro and nanocapsules, which can be found in a solid or semisolid form, likewise allowing the controlled release of active substance from the internal phase to the external phase (13, 14). Among the studies related to encapsulation technique by double emulsion system and complex coacervation applied to foods, aspartame microencapsulation has been studied (15) and other sweeteners (16,17), the same way encapsulation form of casein hydrolyzate (18), microencapsulation of ascorbic acid (19), microencapsulacion carotene β (20), encapsulation of hydrolyzate collagen (21), tuna oil encapsulation (22), peptides casein (23), applications of this technique for the meat industry (24) and microencapsulation of steviol glycosides (25). However, studies for stevia by encapsulation using the mentioned technique have not been reported so the objective of this research was to evaluate the potential of the double emulsion system followed by complex coacervation in stevia encapsulation. The technology efficiency was measured by the size and shape of the capsules, and by measuring the encapsulation capacity.

MATERIALS AND METHODS

Materials

Stevia powder was used (Natursweet ipf, China) as the active compound. Commercial sunflower oil to form the first emulsion (oil phase). Soy lecithin of high viscosity as the lipophilic surfactant. Gelatin 'E441' (Progel, Colombia) and arabic gum (TOUR, Colombia), as components of the wall material capsule.

Elaboration of double emulsion system and stevia encapsulation by complex coacervation

An adaptation of the methodologies used by Rocha *et al.,* 2013 (15) and by Santos *et al.*, 2015 (17) was performed, who encapsulated aspartame and xylitol by double emulsion followed by complex coacervation, respectively. 8 treatments in triplicate were performed, in which stevia was encapsulated up to four concentrations, 3.5; 5; 7.5 and 10% p/p, and two concentrations of wall material, 2.5% and 5% p/p. Additionally, a control treatment was performed, in which stevia was not incorporated. For treatments with wall material concentration of 2.5% it was necessary to use nomenclatures ES1, ES2, ES3, ES4, respectively corresponding to the concentrations 3.5; 5; 7.5 and 10% stevia. For treatments where 5% of concentrations of wall material were used, the corresponding nomenclatures ES5, ES6, ES7 and ES8, were used respectively to the concentrations 3.5; 5; 7.5 and 10% of stevia. Aditionally, a control treatment (ES0) was performed which was developed the same as the ES1 treatment with the difference that the internal solution containing stevia was replaced by distilled water.

For the preparation of active compound, stevia solutions of 3.5, 5, 7.5 and 10% (p/p) were prepared. The solutions were stirred with a magnetic stirrer (MR Hei-Standard, Heidolph) at 28°C, until complete dissolution. As emulsifier lecithin was used in a ratio of 5g of soy lecithin/100g of the total amount of stevia, which corresponded to 0.175g, 0.25g, 0.375g and 0.5g of lecithin, for stevia concentrations 3.5; 5; 7.5 and 10% p/p respectively. Each amount of emulsifier was added to 100 mL of sunflower oil, the mixtures were stirred in Ultra-Turrax (LSK High Shear Mixer, New York) at 3000 rpm for 1 min. To prepare the first emulsion, to 100 mL of sunflower oil plus emulsifier mixture, 100 mL of stevia (at each concentration) were added. Mixtures were stirred in Ultra-Turrax at 5000 rpm for 3 min and the first emulsions were obtained.

The wall material was prepared with arabic gum and gelatin in the ratio 1:1. For the first concentration of wall material 2.5% (p/p), 2.5g of arabic gum and 2.5g of gelatin were weighed, then to 100 mL of distilled water were added each and stirred magnetically to 50°C until completely dissolution and the two solutions were mixed, the final mixture was added to the first emulsion. The same procedure was used for concentration of 5% (p/p).

To prepare the double emulsion and form the coacervate, the first emulsion was added 200 mL of wall material, using a 1:1 ratio in each treatment. The emulsions were stirred in Ultra-Turrax at 3000 rpm for 2 min. Thereby obtaining the double emulsion. The emulsion was adjusted to pH 4.0 with citric acid 0.5% (v/v), causing the formation of the coacervate, which was presented in gel form. Treatments were carried by cooling at 4°C for 24h, time in which complete separation of the two phases and formation of a firm gel was obtained.

The aqueous phase formed due to the desolvation of the polymer, was discarded. Finally, the gels obtained from each treatment were placed in Petri dishes (three dishes per treatment with 6 cm diameter) with approximately 50 mL each. Petri dishes were frozen in ultra-freezer (NEW BRUNSWICK-U101, England) for 6h at -56°C, and they were lyophilized at 0.110 mbar vacuum pressure, and -80°C in the condenser (Labconco, USA).

Morphology of the capsules

Before and after the lyophilization stage, a morphological observation of the capsules was conducted using the methodology Rocha *et al.* (2013) (15), and Santos *et al.* (2015) (17). An optical microscope (Nikon Eclipse E200, TV Lends 0.55X DS, Japan) was used. In order to determine the morphology of the capsules prior to lyophilization, samples of the gel were taken and placed on slides, which was focused with 10X objective. Microscopic images were taken by Optika Vision Pro software. To determine the morphology of the lyophilized capsules, samples were diluted in distilled water to 2% p/p, and then the images were captured, as mentioned above. Images were processed in software image1.48v.

Capsules size

The measurement of capsule size was performed by Zetasizer (Zetasizer Nano S90 Malvern, New York), for capsules before lyophilization, since the device measures sizes of capsules of diluted emulsions (liquid state), and by optical microscopy (Nikon Eclipse e200, TV Lends 0.55X DS, Japan) for lyophilized capsules. The Zetasizer equipment was calibrated by adding 2 ml of distilled water in the reading cell subsequently taken from 1 mL samples of each treatment were diluted in 250 mL of distilled water, 2 mL were placed the solution in a cell reading, and the average values obtained

directly the size of the capsules by malvern Zetasizer software V7.03 (18).

For microscopic measurement, in lyophilized samples, the Optika Vision Pro software was calibrated with micrometric ruler, which allowed making measurements of diameter based on geometry (circular or ovoid). For this study, the capsule diameter was measured in referenced to the circular geometry. For this, 1g of lyophilized capsules in 20 mL of distilled water was dispersed; drops of the solution were taken on a slide and observed by optical microscope, with 10X objective. Statistical analysis graphs were obtained, which were identified as: diameter of capsules before lyophilising (DCBL), diameter of lyophilized capsules (DLC) and wall material (WM).

Fourier´s transformed infrared spectroscopy (FT-IR)

Encapsulation capacity of infrared spectrometry Fourier transform was measured directly in the lyophilized product by using an infrared spectrometer (PIKE MIRacle™ coupled to Spectrum Two FTIR Spectrometer, Madison), which yielded curves with the characteristic peaks of the encapsulated component. From these curves, the software produced a percentage interpreted as encapsulation capacity, which was calculated by the difference between the areas under the curves of the unencapsulated component (stevia) and the encapsulated component (capsules).

In order to do this, 0.1 g of lyophilized capsules was taken and placed on the tip of the equipment reader, the needle was adjusted and the reading was performed using the software PerkinElmer Spectrum™ 10 coupled to the computer. The graphs obtained were analyzed in software Nero Photosnap Wewer, based on wavelengths in the range of 600 cm^{-1} to 4000 cm^{-1}, methodology adapted of Rocha *et al.* (2013) (15).

Water activity (a_w) of the capsules

a_w was measured to lyophilized capsules of each treatment by a team Aqualab (AQUALAB-SERIES 3TE, USA) previously calibrated.

Solubility of capsules

The solubility was measured in lyophilized capsules. 0.5 g samples were taken and diluted in 50 mL of distilled water. The solutions were homogenized in a shaker (MR Hei-Standard. Heidolph) at 50 rpm for 30 min at 28°C. Subsequently, the solution was centrifuged (eppendorf centrifuge 5804R, Germany) to 1369550g during 5 minutes in 25 mL Falcon tubes. Capsules that did not solubilized were separated from the aqueous phase and transferred to a pre dried and weighted porcelain capsule, and subjected to drying at 105°C, until constant weight was reached (15). The solubility was calculated by Equation 1.

$$\text{Solubility (\%)} = (1-(\text{final weight}/\text{initial weight})*100 \qquad \text{Equation 1}$$

Where, initial weight corresponds to the weight of the dried capsules. The final weight corresponds to the weight of the dried not solubilized capsules.

Statistic analysis

A completely randomized multi-level factorial design with 2 factors was used. First factor concentration of stevia, with 4 levels of concentration: 3.5, 5, 7.5 and 10% (p/p). Second factor concentration of the wall material, with two concentration levels 2.5 and 5% (p/p). Treatments were performed in triplicate, for a total of 24 treatments. The response variables were: encapsulation capacity, capsule size (measured in light microscopy and zetaziser), solubility and water activity. Data were analyzed by an ANOVA, using lineal regression model in Minitab software versión17. Tukey test was used in software InfoStat, version 2015, in order to compare treatments. The results were considered statistically significant at $P < 0.05$.

RESULTS

Stevia capsules morphology before and after the lyophilization stage (optical microscopy)

In Figures 1, 2 and 3 the distribution, shape and geometry of the capsules of all treatments can be identified. Figure 1 shows the micrographs of control treatment capsules (ES0), before and after lyophilization. Figure 2 shows the micrographs a, b, c, d, e, f, g and h of the capsules of stevia corresponding to the treatments ES1, ES2, ES3, ES4, ES5, ES6, ES7 and ES8 before lyophilization. In Figure 3 micrographs a’, b’, c’, d’, e’, f’, g’ and h’ of stevia capsules after lyophilization are presented.

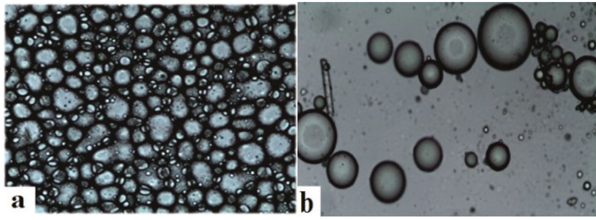

Figure 1. Micrographs of capsules without addition of stevia (control treatments, ES0). **a**) Before lyophilization. **b**) After lyophilization, taken with an optical microscope (TV lends 0.55X DS Nikon-Japan).

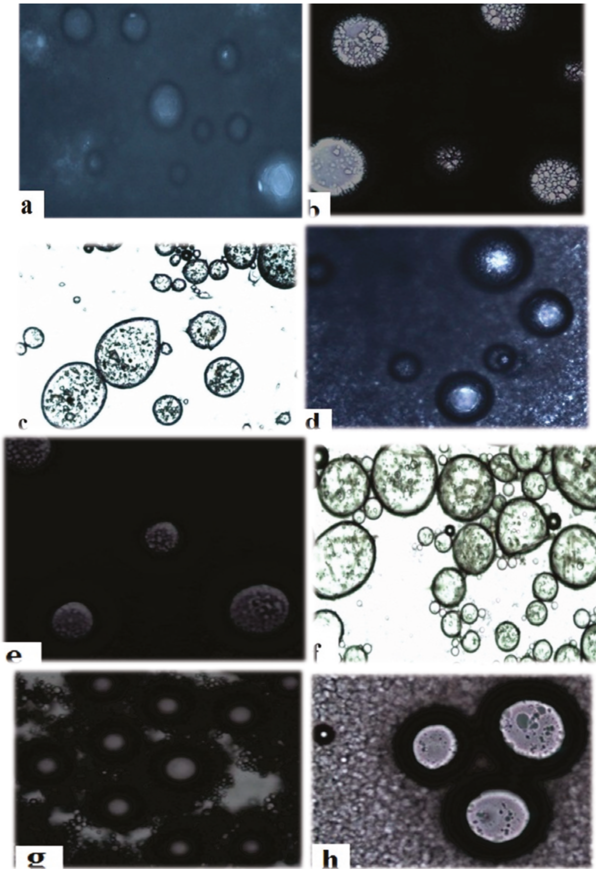

Figure 2. Micrographs of stevia capsules before lyophilization. Taken with optical microscope (TV lends 0.55X DS Nikon-Japan). a, b, c, d, e, f, g and h refer to the ES1, ES2, ES3, ES4, ES5, ES6, ES7 and ES8 treatments (see text nomenclature).

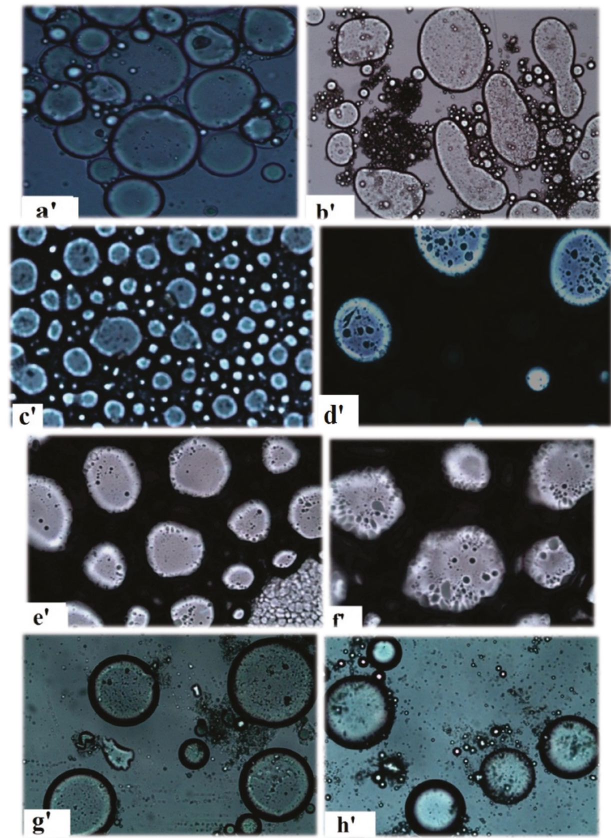

Figure 3. Micrographs of lyophilized and moisturized stevia capsules. Taken with an optical microscope (TV lends 0.55X DS Nikon-Japan). a', b', c', d', e', f', g' and h' refer to ES1, ES2, ES3, ES4, ES5, ES6, ES7 and ES8 treatments (see nomenclature of treatments in text).

Capsule size before and after lyophilization

Table 1, shows the means values (8 treatments each triplicated) capsule size (nm) before and after lyophilization. In figures 4 and 5 the interaction graphs are shown for capsules before and after lyophilization respectively, of the response variable capsule size, in relation to the variation of the stevia (3.5, 5, 7.5 and 10% p/p) and WM concentrations (2.5 and 5% p/p).

Table 1. Capacity of encapsulation (%), average size of capsules before lyophilization (nm) and lyophilized capsules (μm). The values reported correspond to the averages of three repetitions.

Treatments	SCE (p/p)	WMC (p/p)	Encapsulation capacity (%)	Capsule size before lyophilization (nm)	Lyophilized capsule size (μm)
ES1	3,5	2,5	72.060 ± 4.193^{b}	992.997 ± 0.073^{d}	19.393 ± 0.743^{d}
ES2	5,0	2,5	$82.563\pm2.676^{a,b}$	1074.800 ± 0.115^{d}	$26.248\pm2.595^{c,d}$
ES3	7,5	2,5	$80.556\pm4.633^{a,b}$	1429.667 ± 0.065^{d}	33.170 ± 3.683^{c}
ES4	10,0	2,5	60.676 ± 2.421^{c}	$2555.667\pm0.147^{b,c}$	34.998 ± 2.322^{c}
ES5	3,5	5,0	$74.653\pm5.041^{a,b}$	2177.333 ± 0.117^{c}	51.456 ± 2.422^{b}
ES6	5,0	5,0	84.371 ± 4.041^{a}	2941.000 ± 0.171^{b}	61.453 ± 1.652^{a}
ES7	7,5	5,0	51.777 ± 2.267^{c}	4192.000 ± 0.297^{a}	$56.072\pm4.236^{a,b}$
ES8	10	5.0	52.077 ± 4.660^{c}	4326.333 ± 0.292^{a}	62.329 ± 6.654^{a}

Where different letters in the same column indicate statistically significant differences between mean values (P <0.05). The letters a, b, c and d refer to the difference in capsule size, starting from smaller size (a) to larger size (d).

SCE: stevia concentration to encapsulate. WMC: wall material concentration. ES1, ES2, ES3 and ES4: Treatments corresponding to 2.5% of wall material and stevia concentrations of 3.5; 5; 7.5 and 10% p/p. ES5, ES6, ES7 and ES8: Treatments corresponding to 5% of wall material and stevia concentrations of 3.5; 5; 7.5 to 10% p/p.

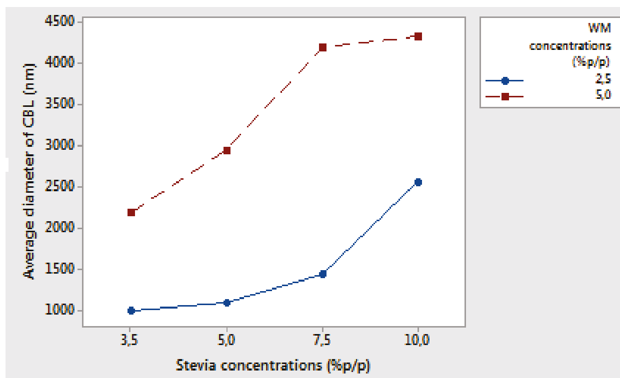

Figure 4. Interaction effect (capsules before lyophilization) among the factors concentration of stevia (3.5; 5; 7.5 and 10% p/p) and concentration of wall material (2.5% and 5% p/p), for the response variable capsule size (see nomenclature graphic text).

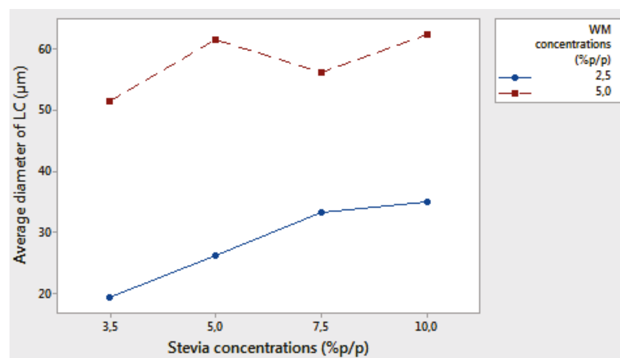

Figure 5. Effect of interaction (lyophilized capsules) between factors concentration of stevia (3.5; 5; 7.5 and 10% p/p) and concentration of wall material (2.5% and 5% p/p) for the response variable size capsules (see nomenclature graphic text).

Fourier´s transformed infrared spectrometry (FT-IR)

In Figures 6 and 7 shows respectively, the graphical representations of the spectra of stevia powder unencapsulated and that of the treatment with higher encapsulation capacity (ES6) obtained in infrared spectrometry.

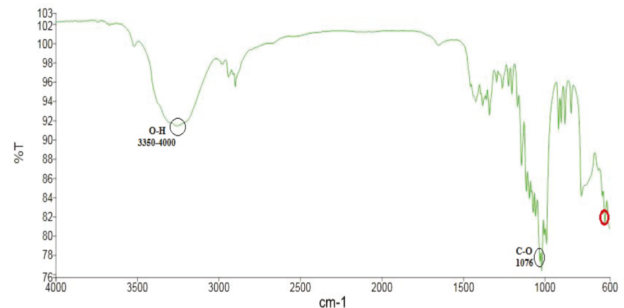

Figure 6. Spectrum obtained in the infrared region of stevia sweetener unencapsulated.

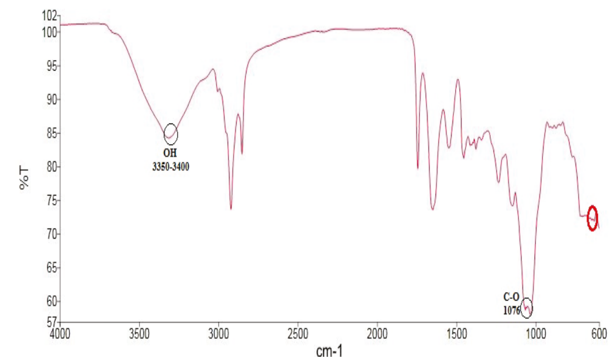

Figure 7. Spectrum obtained in the infrared region of stevia the encapsulated stevia sweetener in ES6 treatment (5% p/p and stevia 5% p/p of the wall material).

Water activity (a_w)

Table 2 shows the means values (8 treatments each one triplicated) of solubility (%) and water activity (aw) after lyophilization, of the 8 treatments and the control treatment ES0.

Different letters in the same column indicate statistically significant differences between mean values (P <0.05). Temperature range in measuring a_w 24.5°C to 26.4°C. (Nomenclature see Table 1).

Table 2. Solubility (%) and water activity of lyophilized microcapsules. The values correspond to the averages of repetitions.

Treatments	Concentration stevia (p/p)	Concentration WM (p/p)	Solubility	a_w
ES0	3,5	2,5	25.715±2.112	0.588±0.019
ES1	3,5	2,5	24.849±1.111^{c}	0.505±0.006
ES2	5,0	2,5	28.268±1.378^{b}	0.564±0.020^{a,b}
ES3	7,5	2,5	34.484±0.481^{a}	0.563±0.013^{a,b}
ES4	10,0	2,5	33.237±1.157^{a}	0.576±0.008^{a}
ES5	3,5	5,0	17.653±0.909^{e}	0.516±0.013^{b,c}
ES6	5,0	5,0	19.723±1.337^{d,e}	0.528±0.031^{a,b,c}
ES7	7,5	5,0	21.809±0.399^{d}	0.495±0.010^{c}
ES8	10	5.0	24.660±0.572^{c}	0.519±0.026^{b,c}

DISCUSSION

Stevia capsules morphology

For the control treatment (Figure 1), in the micrograph a agglomerated homogeneous capsules of defined morphology are observed. In contrast, in micrograph b scattered round capsules of varying diameters are observed. Figure 2 shows capsules before lyophilization, which depict round morphologies (mostly), evidencing the incorporation of small droplets into globules. Each micrograph differs in terms of shape, size and number of capsules, and in turn these are different from the control treatment (Figure 1). This is evidence that the morphology of the capsules is affected by the concentration of stevia, and the concentration of wall materials.

In Figure 3, capsules after lyophilization are observed. In the micrographs a', d', g' and h', few drops incorporated into the capsules are observed. This is because lyophilized capsules were stirred when hydrating causing size reduction of the capsules, which led to the destruction of the globules formed, facilitating the loss of their structure and allowing the release of encapsulated sweetener. Micrographs identified as e', f', g' and h', retained the configuration of the external layer. In treatments with higher concentration of stevia (7.5 and 10% p/p) and low concentration of WM (2.5% p/p), well defined capsules in shape, geometry and distribution were obtained.

Similar behavior was presented at low concentrations of stevia (3.5% and 5% p/p) and high concentrations of WM (5% p/p). Similar micrographs were reported in ascorbic acid coacervate capsules (19) and aspartame (15), likewise, in double emulsion systems stabilized with polysaccharides (26). Furthermore, it is reported that the destruction of the capsules can lead to the release of the active ingredient (27), and this would explain the behavior of treatment ES2.

Capsule size

The sizes of the capsules before lyophilization were the size of nanometers, where the smallest corresponded to the ES1 treatment (Table 1), value that increased as the concentrations of both stevia and concentrations of WM increased, reaching larger diameter ES8 capsules treatment. The size of the lyophilized capsules was micrometric. The treatment group (Tukey) indicated that measurements on optical microscope showed similar behavior to that obtained by Zetasizer. Increase in the size of the lyophilized capsules both in treatments as in the standard sample, is because the capsules before lyophilizing formed a very firm gel, while after lyophilization, the capsules were diluted in distilled water, reason why, capsules increased their volume, dispersed and destroyed because of agitation.

Figures 4 and 5 shows that before and after lyophilization, the capsule size was greater as greater the concentration of stevia (10% p/p), and the greater the concentration of WM (5% p/p) were. ANOVA indicated statistically significant differences between treatments ($P<0.05$), showing greater variation for capsules formed before lyophilization. The graphs (Figure 4 and 5) show that, within the concentrations tested, there was not interaction between factors, however, it is predicted that the diameters of the capsules prior to lyophilization will be greater and presented effect of interaction between the factors for stevia concentration greater

than 10% p/p. From the results it is inferred that the conservation by lyophilization, allows to stabilize the capsules, since the drying process gives the capsules rigidity and firmness, properties essentially strengthened in the wall material, thus allowing to establish a selective diffusion of the encapsulated component. In the encapsulation of flavors and sweeteners, small sizes of capsules provide a delay in the diffusion of the component (14, 17, 28, 29).

The capsule size allows establishing whether or not the capsules will be detectable by the human palate. Martín *et al.* (2009) (30), mention that an average size particle diameter (range of 15 μm to 100 μm) is desirable because larger sizes to 100 μm may be detectable in the mouth, and sizes smaller than 15 μm, do not provide sufficient protection against external factors. Knowing the component to be encapsulated (31,32), and the wall material (12), are indispensable, as they are determining factors in the size of the capsule. Other important factors are speed and stirring time, pH determination of the coacervate and homogenizing mechanism (33-35).

Moreover, Perez *et al.*, (2011) (36), and Zhang *et al.*, (2005) (37), indicate that the variation in the sizes of the capsules formed is mainly due to the degree of interaction between the polymers achieved. In addition, size variation depends on the technique and methodology used for the preparation of capsules (29,38,39). Studies conducted in microencapsulation of functional components report means capsule sizes obtained by double emulsion (before lyophilization) of 84.22 μm to 102.38 μm (15). In the ascorbic acid encapsulation, Comunian *et al.*, (2013) (19), report diameters of capsules before lyophilization of 26.59 μm to 63.11 μm and after lyophilization diameters of 51.67 μm to 83.82 μm.

Fourier´s transformed infrared spectrometry

Table 3 shows the values of wavelengths of the characteristic functional groups of a commercial reference sample stevia powder reported according Bravo *et al.*, (2009) (40). It also shows the spectrum obtained by Moreno & Solano, (2008) (41) in the characterization of stevia extract in plant biomass. In reference to the values of table 3 the points where the presence of the characteristic functional groups of encapsulated stevia identified were located.

Table 3: Fourier´s transformed infrared spectrum in commercial stevia biomass extract.

Spectrum FT-IR of commercial stevia powder stevia (Bravo *et al.*, 2009).		**Spectrum FT-IR of plant biomass of stevia (Moreno & Solano, 2008).**	
Frequency (cm)	Functional Group	Frequency (cm)	Functional Group
3350.07	O-H	3400	OH
2929.98	C-H	2926	C-H (group CH_3)
1727.99	C=O	1627	C-C aromatic
1447.90	C-H	1421	C-H (group CH_2)
1074.52	C-O-C	1076-1005	C-O

Through FT-IR analysis the encapsulation capacity of stevia was obtained, which by ANOVA variance analysis reported that each level assessed presents statistically significant differences for $P<0.05$. The percentages of encapsulation capacity of this study were compared to yield percentages reported in recent studies, finding lower results in the encapsulation of aspartame with 71.70% (15), in the microencapsulation of lycopene with yields from 56.7% to 65.3% (7). Very similar results reported in the microencapsulation of β-carotene with 82.51% (20). Higher yields were obtained in the encapsulation of drugs such as indomethacin (96.3%) and sodium diclofenac (85.66%) (27). While other studies evaluate the encapsulation technique by double emulsion followed by complex coacervation in terms of efficiency (19).

Martín *et al.*, 2009 (30), mention that it is possible to obtain round microcapsules with sizes up to 4 μm and with a 90% of the active material incorporated at the end of the encapsulation process. Madene *et al.*, 2006 (28) indicate that between encapsulation processes, the technique of complex coacervation allows to incorporate functional compounds in microcapsules in order to provide protection to the encapsulated principle, hence the importance of encapsulating stevia in order to conceal the bitter taste that this component presents (6).

The spectra in the infrared region may vary according to the main components present in the extracts of stevia, however, peaks have been identified representing the presence of some functional groups of stevia for certain wavelengths (41), as shown in table 3. Thus for the present study was obtained the figure 7, in which the O-H functional group was identified to a range of wavelength 3350 cm^{-1} to 3400 cm^{-1} as reported by Moreno & Solano, 2008 (41) and Bravo *et al.*, 2009 (40), similarly, the representation of the C-O group in the region of 1076 cm^{-1} was obtained, in which for a more pronounced peak reference is made to the presence of stevioside, while if the peak is shorter is because of the presence of rebaudioside A. The specification is met when the values are reported in % of transmittance (42). Furthermore, the formation of the located peaks between 1500 cm^{-1} to 1640 cm^{-1} is noticeable, which represent amides groups, formed by interaction between the carboxyl groups of the gum and the amino group of the protein, thus confirming the formation of the coacervate (15). Finally, the point marked in the 650 cm^{-1} region is mentioned to be part of any component of the stevia but has not yet been identified with certainty the type of glucoside present (42). In Figure 6 the representation of the spectrum for stevia unencapsulated was obtained which was taken as reference and served as the base to identify analogies regarding Figure 7.

Water activity (a_w)

In the variable a_w no significant statistically differences between treatments ES2, ES3 and treatments ES5, SS8 ($P < 0.05$) were shown. As for the values reported in Table 2, it can be inferred that lyophilized microcapsules reached a range a_w between 0.495 and 0.576, which shows that the microcapsules of stevia are stable against degradation mechanisms, since it is reported that with a_w minor or equal to 0.6, microbiological stability is guaranteed (19). Very close values were obtained close to the one reported in ascorbic acid encapsulation (0.318-0.515) and lower values in the encapsulation of lycopene (0.26 to 0.29) (7).

Microcapsules solubility

The property of solubility in lyophilized microcapsules was evaluated in order to establish their behavior when added in water, as shown in Table 2, statistically significant differences ($P<0.05$) between concentration levels of stevia and in the concentration levels of WM were obtained. Furthermore, there was a significant increase in the solubility as the concentration of stevia increased, which is due to the high degree of solubility of the stevia in its pure state (4), so that greater quantities of stevia favor the interaction with hydrophilic molecules and thereby improves its affinity with the aqueous medium, however, the microcapsules corresponding to treatments with a higher concentration of WM (ES5, ES6, ES7 and ES8) are less soluble which is related to encapsulation capacity when lower solubility greater capacity, as reported in studies of encapsulation of casein hydrolyzed (18) and encapsulation of xylitol (17). Moreover, Saravanan & Rao, (2010) (27) mention that the active components with high water solubility have less stability and rapid release compared to encapsulated insoluble compounds by complex coacervation. In the present study, it was found that the treatments ES5, ES6, ES7 and ES8 were less soluble in compared to the control and in turn to the ES1, ES2, ES3 and ES4 treatments. The solubility behavior percentage varied in relation to the concentration of WM, because the viscosity properties, solubility and emulsifying properties are favored when using gums and gelatins together and at high concentrations (28). In addition, interactions between biopolymers determine to some extent the stability of microcapsules formed by complex coacervation (29).

CONCLUSIONS

The morphological characteristics of the microcapsules, analysis by infrared spectrometry and properties such as capsule size, solubility and water activity allows to conclude that it is possible to encapsulate stevia by applying the double emulsion system (W/O/W) followed by complex coacervation. The treatment with morphological characteristics which showed the formation of droplets within the microcapsules and higher encapsulation properties capacity and lower solubility was the ES6 treatment, which allows concluding that low concentrations of stevia and concentration of 5% p/p of wall material, allows to decrease solubility and increase the content of stevia into capsules, favoring the incorporation of the sweetener.

CONFLICTS OF INTEREST

The authors declare not to have conflict of interest whit this research.

AUTHORS' CONTRIBUTIONS

Conception and design of study: Serna C. Liliana and Ayala A. Alfredo. Acquisition, analysis and interpretation of data: Micanquer C. Adriana.

REFERENCES

1. Bearth A, Cousin M, Siegrist M. The consumer's perception of artificial food additives: Influences on acceptance, risk and benefit perceptions. *Food Qual Prefer.* 2014 May 14; 38, 14-2. http://doi.org/10.1016/j.foodcont.2016.01.044
2. Nakonieczna A, Paszkowski B, Wilczek A, Szypłowska A, Skierucha W. Electrical impedance measurements for detecting artificial chemical additives in liquid food products. *Food Control*. 2016 Jan 5; 66(1), 116-129. http://doi.org/10.1016/j.foodqual.2014.05.008
3. Zhang S, Kumar A, Kutowy O. Membrane-based separation scheme for processing sweeteners from stevia leaves. *Food Res Int.* 2000 Jan 6; 33(7), 617-620.
4. Lemus R, Vega A, Zura L, Kong A. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food Chem.* 2011 Nov 16; 132(3), 1121-1132. http://doi.org/10.1016/j.foodchem.2011.11.140
5. Sylvetsky A, & Rother K. Trends in the consumption of low-calorie sweeteners. *Physiol. Behav.* 2016 Oct 1; 1, 1-17. http://doi.org/10.1016/j.physbeh.2016.03.030
6. Marcinek K, Krejpcio Z. Stevia rebaudiana bertoni-chemical composition and functional properties. *Acta Sci. Pol. Technol. Aliment.* 2015. 14(2), 145-152. http://doi.org/10.17306/J.AFS.2015.2.16
7. Silva D, Favaro-Trindade C, Rocha A, Thomazini M. Microencapsulation of lycopene by gelatin-pectin complex coacervation. *J. Food Process. Pres.* 2011 Jun 21; 36(2), 185-190. http://doi.org/10.1111/j.1745-549.2011.00575.
8. Li B, Jiang Y, Liu F, Chai Z, Li Y, Leng X. Synergistic effects of whey protein-polysaccharide complexes on the controlled release of lipid-soluble and water-soluble vitamins in W1/O/W2 double emulsion systems. Int. J. Food Sci. Tech. 2011 Nov 11; 47, 248-254. http://doi.org/10.1111/j.1365-621.2011.02832.
9. Kanai T, Tsuchiya M. Microfluidic devices fabricated using stereolithography for preparation of monodisperse double emulsions. *Chm. Eng. J.* 2016 Apr 15; 290, 400-404. http://doi.org/10.1016/j.cej.2016.01.064
10. Assadpour E, Maghsoudlou Y, Jafari S, Ghorbani M, Aalami M. Optimization of Folic Acid Nano-emulsification and Encapsulation by Maltodextrin-Whey Protein Double Emulsions. *Int. J. Biol. Macromol*. 2016 May; 86, 197-207. http://doi.org/10.1016/j.ijbiomac.2016.01.064
11. Schmidt U, Bernewitz R, Guthausen G, Schuchmann H. Investigation and application of measurement techniques for the determination of the encapsulation efficiency of O/W/O multiple emulsions stabilized by hydrocolloid gelation. *Colloid Surface A.* 2015 Jun 20; 475(1), 55-61. http://doi:10.1016/j.colsurfa.2014.12.040
12. Sandoval A, Rodriguez E, Ayala A. Encapsulation of additives for the food industry. *Ingenieria Y Competitividad.* 2004 Apr 30; 5(2), 73-83. http://bibliotecadigital.univalle.edu.co/handle/10893/1561
13. Shimokawa K, Saegusa K, Wada Y, Ishii F. Physicochemical properties and controlled drug release of microcapsules prepared by simple coacervation. *Colloid Surfaces B.* 2013 Apr 1; 104, 1-4. http://doi.org/10.1016/j.colsurfb.2012.11.036
14. Jiménez-Colmenero F. Multiple emulsions; Bioactive compounds and functional foods. Hosp. Nutr. 2013 May 15; 28(5), 1413-1421. http://doi.org/10.3305/nh.2013.28.5.6673
15. Rocha G, Bozza F, Thomazini M, Bolini H, Fávaro C. Microencapsulation of aspartame by double emulsion followed by complex coacervation to provide protection and prolong sweetness. *Food Chem.* 2013 Aug 15; 139(1), 72-78. http://doi.org/10.1016/j.foodchem.2013.01.114
16. Santos M, Carpinteiro D, Thomazini M, Rocha G, Da Cruz A, Rodriges C, Favaro C. Coencapsulation of xylitol and menthol by double emulsion followed by complex coacervation and microcapsule application in chewing gum. *Food Res Int.* 2014 Oct 22; 66(1), 454-462. http://doi.org/10.1016/j.foodres.2014.10.010
17. Santos M, Bozza F, Thmazini M, Favaro C. Microencapsulation of xylitol by double emulsion followed by complex coacervation. *Food Chem.* 2014 Aug 21; 171(1), 32-39. http://doi.org/10.1016/j.foodchem.2014.08.093
18. Mendanha D, Molina S, Favaro C, Mauri A, Monterrey E, Thomazini M. Microencapsulation of casein hydrolysate by complex coacervation with SPI/pectin. *Food Res Int.* 2009 May 20; 42(8), 1099-1104. http://doi.org/10.1016/j.foodres.2009.05.007
19. Comunian T, Thomazini M, Alves A, Matos F, de Carvalho J, Favaro C. Microencapsulation of ascorbic acid by complex coacervation: Protection and controlled release. *Food Res Int.* 2013 Mar 16; 52(1), 373-379. http://doi.org/10.1016/j.foodres.2013.03.028
20. Jain A, Thakur D, Ghoshal G, Katare O, Shivhare U. Characterization of microcapsulated β-carotene formed by complex coacervation using casein and gum tragacanth. *Int. J. Biol. Macromol.* 2016 Jun 31; 87, 101-113. http://doi.org/10.1016/j.ijbiomac.2016.01.117
21. Ocak B. Complex coacervation of collagen hydrolysate extracted from leather solid wastes and chitosan for controlled release of lavender oil. *J. Environ. Manage.* 2012 Jun 15; 100(1), 22-28. http://doi.org/10.1016/j.jenvman.2012.01.026
22. Wang B, Adhikari B, Barrow C. Optimisation of the microencapsulation of tuna oil in gelatin-sodium hexametaphosphate using complex coacervation. *Food Chem.* 2014 Feb 24; 158, 358-365. http://doi.org/10.1016/j.foodchem.2014.02.135
23. Giroux H, Robitaille G, Britten M. Controlled release of casein-derived peptides in the gastrointestinal environment by encapsulation in water-in-oil-in-water double emulsions. *LWT-Food Sci. Tech.* 2016 Jun; 69, 225-232. http://doi.org/10.1016/j.lwt.2016.01.050
24. Serdaroğlu M, Öztürk B, Urgu M. Emulsion characteristics, chemical and textural properties of meat systems produced with double emulsions as beef fat replacers. *Meat Sci.* 2016 Jul; 117, 187-195. http://doi.org/10.1016/j.meatsci.2016.03.012
25. Chranioti C, Chanioti S, Tzia C. Microencapsulation of steviol glycosides (Stevia rebaudiana Bertoni) by a spray drying method–Evaluation of encapsulated products and prepared syrups. *Int J. Food Studies.* 2015 Oct 18; 4(2), 212-220.
26. Benna Z, Kbir A, Trabelsi A, Grossiord J. Stabilisation of W/O/W double emulsion by polysaccharides as weak gels. *Colloid Surfaces A.* 2007 Aug 17;. 316(1), 46-54. http://doi.org/10.1016/j.colsurfa.2007.08.019.
27. Saravanan M, Rao K. Pectin-gelatin and alginate-gelatin complex coacervation for controlled drug delivery: Influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. *Carbohyd. Polym.* 2010 May; 80(3), 808-816. http://doi.org/10.1016/j.carbpol.2009.12.036
28. Madene A, Jacquot M, Scher J, Desobry S. Flavour encapsulation and controlled release-A review. *Int. J. Food Sci Tech.* 2005 Dec 1; 41(1), 1-21. http://doi.org/10.1111/j.1365-621.2005.00980.
29. Jun-xia X, Hai-yan Y, Jian Y. Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic. *Food Chem.* 2010 Oct 13; 125(4), 1267-1272. http://doi.org/10.1016/j.foodchem.2010.10.063
30. Martín M, Morales M, Gallardo V, Ruiz M. Microencapsulation techniques: A proposal to microencapsulate probiotics. Ars Pharmaceutica. 2009 Jun 18; 50(1), 43-50.

31. McClements D, Decker E, Weiss J. Emulsion-based delivery systems for lipophilic bioactive components. *J. Food Sci*. 2007. 72(8), 109-124. http://doi.org/10.1111/j.1750-841.2007.00507.
32. McClements D, Decker E, Park Y, Weiss J. Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Crit. Rev. Food Sci.* 2009 May 28; 49(6), 577-606. http://doi.org/10.1080/10408390902841529
33. Lobato-Calleros C, Rodriguez E, Sandoval O, Vernon E, Alvarez J. Reduced-fat white fresh cheese-like products obtained from W1/O/W2 multiple emulsions: Viscoelastic and high-resolution image analyses. *Food Res Int.* 2006 Jan 9; 39(6), 678-685. http://doi.org/10.1016/j.foodres.2006.01.006
34. Mayer S, Weiss J, McClements D. Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: Factors influencing droplet size and stability. *J. Colloid Interf. Sci.* 2013 Apr 4; 402(1), 122-130. http://doi.org/10.1016/j.jcis.2013.04.016
35. Zhang R, Zhang Z, Zhang H, Decker A, McClements D. Influence of emulsifier type on gastrointestinal fate of oil-in-water emulsions containing anionic dietary fiber (pectin). *Food Hydrocolloid*. 2014 Nov 21; 45(1), 175-185. http://doi.org/10.1016/j.foodhyd.2014.11.020
36. Perez J, Barrios E, Roman A, Pedroza R. Interaction of Mesquite Gum-Chitosan At the Interface and Its Influences on the Stability of Multiple Emulsions W1/O/W2. J. Mex. Eng.Chem. 2011. 10(3), 487-499. http://doi.org/10.1016/S0963-9969(00)00098-3
37. Zhang J, Chen D, Wang S, Zhu K. Optimizing double emulsion process to decrease the burst release of protein from biodegradable polymer microspheres. *J. Microencapsul.* 2004 Dec 17;. 22(4), 413-422. http://doi.org/10.1080/02652040500098994
38. Charcosset C. Preparation of emulsions and particles by membrane emulsification for the food processing industry. *J. Food Eng.* 2009 Jun; 92(3), 241-249. http://doi.org/10.1016/j.jfoodeng.2008.11.017.
39. Lamba H, Sathish K, Sabikhi L. Double Emulsions: Emerging Delivery System for Plant Bioactives. *Food Bioprocess Tech.* 2015 Apr; 8(4), 709-728. http://doi.org/10.1007/s11947-014-1468-6
40. Bravo M, Ale N, Rivera D, Huamán J, Delmás D, Rodríguez M, Bautista M. Chemical characterization of *Stevia Rebaudiana*. J. *Per. Chem. Eng. Chem* 2009. 12, 5-8. http://doi.org/10.1016/j.colsurfb.2012.11.036
41. Moreno L, Solano E. Utilización del subproducto vegetal generado en la Elaboración de edulcorantes de stevia como material de relleno reforzante en elastómeros de poliuretano a partir de aceite de *Higuerilla.* In tesis doctoral. Santander-Colombia. 2008. 1-39 pp.
42. Giraldo C, Martín L, Habeych D. Obtaining sweeteners from *Stevia Rebaudiana Bertoni*. *CENIC Biol. Sci.* 2005. 36(1), 1-9. http://www.redalyc.org/pdf/1812/181220525077.pdf