



## ENCAPSULATION OF ASCORBIC ACID INTO CALCIUM ALGINATE MATRICES THROUGH COACERVATION COUPLED TO FREEZE-DRYING

## ENCAPSULACIÓN DE ÁCIDO ASCÓRBICO EN MATRICES DE ALGINATO DE CALCIO POR COACERVACIÓN Y LIOFILIZACIÓN

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### Abstract

The aim of this work was to study the stability of ascorbic acid (AA) after encapsulation into calcium alginate matrixes by coacervation through: a) falling drop by dipping AA-sodium alginate solutions into  $\text{CaCl}_2$  solutions (IAA method), and by atomizing the AA-sodium alginate solutions into  $\text{CaCl}_2$  solutions (SAA method), followed by freeze-drying of the obtained capsules. Sorption isotherms for both types of products were determined at the water activity ( $a_w$ ) range of 0.103-0.892, at 15, 25 and 35 °C, by using the gravimetric method with saturated salt. The percentage of retention of AA was determined, after 15, 30, 45 and 60 days of storage at 25 °C. SEM micrographs of the products were obtained and the volume of micropores was also calculated. The minimum integral entropy of the water molecules occurred at  $a_w = 0.234$  in the falling drop method powders, whereas this parameter was observed at  $a_w = 0.706$  for the atomised one. For both types of products, the maximum value of AA retention was observed at the  $a_w$  corresponding to the minimum integral entropy which occurred at higher moisture contents for SAA than for IAA materials.

**Keywords:** ascorbic acid, encapsulation, minimum integral entropy, water sorption, storage stability.

### Resumen

El objetivo de este trabajo fue estudiar la estabilidad del ácido ascórbico (AA) encapsulado en una matriz de alginato de calcio por coacervación, utilizando dos métodos: a) goteo por inmersión de una solución de AA-Alginato de sodio en una solución de  $\text{CaCl}_2$  (IAA), y atomización de una solución de AA-Alginato de sodio en una solución de  $\text{CaCl}_2$  (SAA), seguido de secado por liofilización. Las isoterms de sorción de ambos productos se determinaron en intervalo de actividad de agua ( $a_w$ ) de 0.103-0.892, a 15, 25 y 35 °C, utilizando el método gravimétrico con sales saturadas. El porcentaje de retención de AA se determinó, después de 15, 30, 45 y 60 días de almacenamiento a 25°C. Se obtuvieron micrografías por SEM y se calculó el volumen de microporos. La mínima entropía integral se obtuvo a una  $a_w = 0.234$  en los productos obtenidos por goteo, mientras que en el polvo obtenido por atomización se encontró a una  $a_w = 0.706$ . En ambos productos, el valor máximo de la retención de AA se observó en la  $a_w$  correspondiente a la mínima entropía integral, la cual se encontró en contenidos de humedad más altos para SAA que en IAA.

**Palabras clave:** ácido ascórbico, encapsulación, mínima entropía integral, sorción de agua, estabilidad durante el almacenamiento.

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## 1 Introduction

The loss of nutritional quality during processing and storage of food commodities has become a major issue in the production chain. Vitamins are important components of foods and a considerable number of works aiming to preserve them throughout the processing chain have been carried out (Kirby *et al.*, 1991; Lee and Coates, 1999; Uddin *et al.*, 2001; Klimezak *et al.*, 2007; Montes *et al.*, 2007; Pulido and Beristain, 2010). Vitamin C has been frequently selected as an index of nutrient quality due to its thermolabile nature in comparison to other nutrients in different processed foods (Erentuk *et al.*, 2005). The richest sources of vitamin C are fruits and vegetables such as guava, papaya, orange, peppers, kiwi fruit, strawberries, etc., which provide over 90% of vitamin C in the human diet (Erentuk *et al.*, 2005). Also, a high intake of vitamin C has been suggested, based on biochemical, epidemiological and clinical studies, which support a recommended daily intake of vitamin C of around 100 to 120 mg per day aiming to reduce risks of heart diseases, brain stroke and cancer (Olivier, 2004; Klimezak, 2007). Supplementation of foods with L-ascorbic acid increases the technological quality and nutritional value of food products (Burdurly *et al.*, 2006; Lee and Coates, 1999). Ascorbic acid is stable in powder form but this stability decreases during processing. The main factors that may affect the loss of ascorbic acid, include temperature, concentration of salt and sugar, pH, oxygen and exposure to light, metal catalysts, ratio of ascorbic to dehydroascorbic acid, and microbial load of the food (Zerdin *et al.* 2003), all these factors can produce large losses of vitamin C and changes in colour from white to yellow (Wilson and Shah, 2007). Vitamin C is one of the most used nutritional indicators during processing and storage of foods given its vulnerability to chemical and enzymatic oxidations, and high solubility in water (Martins and Silva, 2004). Many studies have been conducted to determine the most stable form of this vitamin during processing and a good option to protect it against degradation is by microencapsulation, which is defined as a process in which the bioactive substances (flavours, vitamins, antioxidants, antimicrobial essential oils) are introduced into a relatively small matrix or wall materials (Lekago and Dunford, 2010).

Microencapsulation allows reducing volatility, hygroscopicity and reactivity thus increasing product stability under adverse environmental conditions

(Favaro *et al.*, 2010). Microcapsules help food materials to resist processing and packaging conditions to improve flavour, odour, stability, nutritional value and appearance (Montes *et al.*, 2007). Different techniques have been developed to obtain microcapsules such as spray drying, extrusion, coacervation, liposome formation, freeze-drying, chilling or freezing, etc. (Gouin, 2004; Madene *et al.*, 2006; Quintanilla-Carvajal *et al.*, 2011). Drying can be defined as a process to remove water due to the simultaneous transfer of heat and mass between the product and a stream of hot air, which is usually caused by temperature and air convection forces (Waewsak *et al.*, 2006; Arabhosseini *et al.*, 2009; Gazor and Mohsenimanesh, 2010). Freeze-drying of biological materials is also used for water removal with a minimum of associated heat damage to the solid matrix and is based on the sublimation of the ice fraction of the product (Sagar and Suresh, 2009). Mass transfer occurs by diffusion of vapour through the dry layer of the specimen under the action of a pressure difference (Orrego, 2003).

A suitable level of moisture for storage is that corresponding to the minimum integral entropy of the system as proposed by Beristain and Azuara (1990). At this moisture content, water is less available for deteriorating reactions (Beristain *et al.*, 2002, Azuara and Beristain 2006, Dominguez *et al.*, 2007; Gutierrez *et al.*, 2007; Pascual *et al.*, 2010, Flores *et al.*, 2009). The results obtained by Azuara and Beristain (2006) also showed that the moisture content value in the minimum entropy was found at the moisture content corresponding to the volume of micropores as calculated by means of the model of Dubinin-Radushkevich (Fletcher and Thomas, 2000).

## 2 Materials and methods

### 2.1 Materials

#### 2.1.1 Chemicals

Sodium alginate (Grindsted, Mexico), calcium chloride (Sigma-Aldrich, Mexico) and ascorbic acid (Sigma-Aldrich, Mexico) were used for the encapsulation experiments.  $P_2O_5$ , LiCl,  $KC_2H_3O_2$ ,  $MgCl_2$ ,  $K_2CO_3$ ,  $Mg(NO_3)_2$ ,  $NaNO_3$ , NaCl and KCl (Sigma-Aldrich, Mexico) were used for the determination of sorption isotherms.

## 2.2 Methods

### 2.2.1 Encapsulation by coacervation, falling drop method (IAA)

A solution of sodium alginate 2 % w/v was prepared by dispersing, at room temperature, the sodium alginate powder in deionized water and stirring speedily. A mixture 1:1 was prepared by adding this solution into a 1 % w/v ascorbic acid solution and homogenising for 20 minutes (Blade Homogenizer, Braun, Mexico). Also, a 2 % w/v solution of calcium chloride was prepared by dispersing the salt in deionised water. To carry out the encapsulation, the mixture sodium alginate-ascorbic acid was added drop by drop into the calcium chloride solution by using a 5 mL pipette from a height of 10.0 cm at which good penetration of the droplet into the liquid was observed (Flores-Andrade, 2010). The ratio between the mixture and the calcium chloride solution was 1.5. At this ratio, there was sufficient liquid ( $\text{Ca}^{2+}$ ) to promote coacervation (Flores-Andrade, 2010). The excess of calcium chloride was removed by vacuum filtering through a  $< 2\mu\text{m}$  Whatman filter paper and washing once with deionized water. The capsules so formed were freeze-dried at  $-50\text{ }^\circ\text{C}$  and 0.01 mbar (Labconco Lyph Look 4.5, USA).

### 2.2.2 Encapsulation by coacervation, spraying method (SAA)

The same solutions (alginate, ascorbic acid and calcium chloride) were prepared as described above. To carry out the encapsulation the sodium alginate-ascorbic acid mixture was fed (0.542 L/h) by using a peristaltic pump (Thermo Scientific) to a two fluid pneumatic nozzle (Spraying Systems Co., 1/8JJ, USA), previously set to 240 kPa spraying air pressure and atomised in the calcium, chloride solution. The tip of the nozzle was located 10.0 cm above the surface of the liquid. The volume ratio between the alginate-ascorbic acid and the calcium chloride solution was as in the IAA method, 1.5. The excess of calcium chloride was removed by filtration through a  $< 2\mu\text{m}$  Whatman filter paper under vacuum and washing once with deionised water. The gel formed was then freeze-dried at  $-50\text{ }^\circ\text{C}$  and 0.01 mbar (Labconco Lyph Look 4.5, USA).

### 2.2.3 Sorption isotherms

Sorption isotherms were constructed by using the gravimetric method proposed by Lang *et al.* (1981).

1-2 g of the freeze dried powders were placed into desiccators containing  $\text{P}_2\text{O}_5$  for one week to reduce the moisture content to nearly zero, after this time, the dry-basis weight was recorded. As total, 8 saturated salt solutions were prepared with water activities in the range of 0.103 to 0.892 according to Labuza *et al.* (1985). The samples were placed in equilibration cells which contained the saturated salts at constant temperatures of 15, 25 and  $35\text{ }^\circ\text{C}$ . Every three days the samples were weighed on an analytical balance (OHAUS Analytical Plus, AP210S, USA) until constant weight was achieved. The equilibrium moisture content on dry-basis was determined for each water activity. Isotherms were determined by duplicate and fitted by using KaleidaGraph software from Synergy software, Reading PA, USA to the model of Guggenheim-Anderson-De Boer (GAB) (Weisser, 1985):

$$M = \frac{M_0CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \quad (1)$$

Where  $M$  = equilibrium moisture content (g of water/100 g of dry solid),  $a_w$  = water activity,  $M_0$  = monolayer moisture content,  $C$  and  $K$  are constants related to temperature as follows:

$$C = c \exp\left[\left(\frac{h_m - h_n}{RT_a}\right)\right] \quad (2)$$

$$K = k \exp\left[\left(\frac{h_n - h_l}{RT_a}\right)\right] \quad (3)$$

Where  $C$  and  $K$  = entropic accommodation factors,  $h_m$ ,  $h_n$  and  $h_l$  = molar sorption enthalpies of the monolayer, the multilayers on top of the monolayer, and the bulk liquid, respectively (in J/mol); while  $R$ (J/mol K) is the ideal gas constant and  $T_a$ (K) is the absolute temperature.  $C$ ,  $K$  and  $M_0$ , were obtained from the fitted GAB model data and by using KaleidaGraph software from Synergy software, Reading PA, USA.

### 2.2.4 Calculation of thermodynamic properties

The integral enthalpy changes  $(\Delta H_{int})_T$  (J/mol) of the samples at the different water activities, were determined using the equation of Othmer (1940).

$$\frac{d \ln P_v}{d \ln P_v^0} = \frac{H_v(T)}{H_v^0(T)} \quad (4)$$

Where:  $P_v$  (atmospheres or atm.) is the vapour pressure of water in the sample;  $P_v^0$  (atm) is the vapour pressure (atm) of pure water at the temperature of sorption;  $H_v(T)$  (J/mol) is the integral molar heat of sorption, and  $H_v^0(T)$ (J/mol) is the heat of condensation

of pure water. Since all these terms are temperature dependent, the equation can be integrated as follows:

$$\ln P_v = \left[ \frac{H_v(T)}{H_v^0(T)} \right]_{\phi} \ln P_v^0 + A \quad (5)$$

Where  $A$  is the adsorption constant and  $\phi$  (J/mol) is the pressure of diffusion or surface potential. A plot of  $\ln P_v$  versus  $\ln P_v^0$  gives a straight line if the ratio  $H_v(T)/H_v^0(T)$  is constant within the range of temperatures used.

The molar integral enthalpy  $(\Delta H_{int})_T$  can be calculated using Eq. (6), at a constant pressure of diffusion (Beristain *et al.*, 1994).

$$(\Delta H_{int})_T = \left[ \frac{H_v(T)}{H_v^0(T)} - 1 \right]_{\phi} H_v^0(T) \quad (6)$$

$$\phi = \mu_{ap} - \mu_a = RT \frac{W_{ap}}{W_v} \int_0^{a_w} M d \ln(a_w) \quad (7)$$

Where  $\mu_a$  (J/mol) is the chemical potential of the adsorbent participating in the condensed phase,  $\mu_{ap}$  (J/mol) is the chemical potential of the pure adsorbent,  $W_{ap}$  (g/mol) is the molecular weight of the adsorbent, and  $W_v$  (g/mol) is the molecular weight of water.

By calculating  $H_v(T)/H_v^0(T)$  from Eq. (5) and substituting it into Eq. (6) it is possible to calculate the integral enthalpy at different temperatures, provided that a good means of estimating  $H_v^0(T)$  is available, such as that proposed by Wexler (1976):

$$H_v^0(T) J/mol^{-1} K^{-1} = 6.15 \times 10^4 - 94.14T + 17.74 \times 10^{-2} T^2 - 2.03 \times 10^{-4} T^3 \quad (8)$$

By using the obtained values for  $(\Delta H_{int})_T$ , it was possible to estimate the changes in the integral molar entropy  $(\Delta S_{int})_T$  by means of the following equation:

$$(\Delta S_{int})_T = S_s - S_L = -\frac{(\Delta H_{int})_T}{T} - R \ln a_w \quad (9)$$

Where  $S_s = S/N_1$  (J/mol K) is the integral entropy of water adsorbed in the material;  $S$  (J/mol K) is the total entropy of water adsorbed;  $N_1$  is the moles of water adsorbed in the sample and  $S_L$  (J/mol K) is the molar entropy of pure liquid water in equilibrium with vapour.

### 2.2.5 Calculation of moisture content corresponding to the volume of micropores

The Dubinin-Radushkevich model is still the most widely used for the fractional pore filling of the

micropores (Sonwane and Bhatia, 2000). Therefore, the moisture content corresponding to the micropore volume ( $n_o$ ) was obtained using the Dubinin-Radushkevich equation (Fletcher and Thomas, 2000; Azuara and Beristain, 2006) which assumes a physical adsorption and compensation effects for the kinetics of adsorption /desorption of gases/vapors into the micropores and which allows, in its linear form, the evaluation of the volume of micropores through the intercept of the straight line obtained by plotting  $\log_{10} n_o$  vs.  $\log^2 (P_v^0/P_v)$ . According to Fletcher and Thomas, (2000),  $n_o$  is the parameter that best physically characterises a microporous material.

$$\log_{10} n = \log_{10} n_o - B \log_{10}^2 \left( \frac{P_v^0}{P_v} \right) \quad (10)$$

Where  $n$  (g of water/100g of dry solids) is the amount of moisture adsorbed;  $n_o$  (g water / 100 g dry solids) is the amount of adsorbed moisture corresponding to the volume of micropores, and  $B$  is a constant related to the microporous structure of the adsorbent.

### 2.2.6 Determination of ascorbic acid (AA)

The determination of ascorbic acid was made by the second order derivative spectrophotometric method (Pfundt *et al.*, 2003), by using a diode array spectrophotometer (Agilent 84538); 25 mg samples were placed into 50 mL volumetric flasks and solubilised with HCl 0.1 M; the solutions were filtered and centrifuged at 4000 rpm for 15 minutes with the purpose of removing solid material and obtaining a homogeneous solution. 1 mL aliquots were taken from these solutions and diluted 50 times with HCl 1 M and their absorbance registered in the spectrophotometer at 267 nm. A standard curve was used to calculate the content of ascorbic acid in the samples which was reported as mg of ascorbic acid per g of sample.

To determine the stability of AA during storage, samples were stored in an oven (LAB LINE, AMBI-HI-LO, USA) at 25 °C and at the range of  $a_w$  values used for constructing the sorption isotherms. The AA content was evaluated immediately after preparing the capsules (time zero) and at 15, 30, 45 and 60 days of storage.

### 2.2.7 SEM micrographs

The microstructure of the capsules surface stored at the  $a_w$  value corresponding to the minimum integral entropy was examined by using an Environmental Scanning Electron Microscope (XL-30, Philips, USA)

working at 25 kV and different amplifications and by using a BSE detector.

### 3 Results and discussion

#### 3.1 Sorption behaviour of capsules

Figure 1, shows the experimental points (markers) and the fitted points, using the GAB equation (lines) of water vapour adsorption isotherms at 25 °C for the capsules containing ascorbic acid encapsulated using coacervation by the falling drop (IAA) and by the spraying methods (SAA). This temperature was chosen for evaluating ascorbic acid stability. The isotherms of SAA showed relatively high adsorption capacities which caused the presence of a curvature at the start of the adsorption line, therefore, these materials may be considered as hydrophilic. Despite their adsorption behaviour at low  $a_w$ , the materials did not show large changes in moisture content as  $a_w$  increased, which indicated increased stability (Labuza, 1980). For IAA samples, isotherms showed marked changes in moisture content as  $a_w$  increased and no curvature at the beginning of the sorption process. To determine the extent of similarity between the results from the two repetitions at each sorption temperature, a t-test was performed at  $\alpha = 0.05$ , by using the software Sigma Stat 3.5 (Systat Software Inc., USA). There was not a significant difference between moisture contents at the same  $a_w$  ( $p = 0.710$ ).

The estimated moisture content at the various water activity values, were obtained by fitting experimental data to the GAB equation which adequately represented sorption data in the whole range of  $a_w$  studied. Table 1 shows the values of  $K$  and  $C$  (constants related to energy) and  $M_0$  (monolayer) for IAA and SAA. From the constant  $C$ , it is possible to classify the type of physisorption according to Brunauer *et al.* (1940). It is possible to observe that IAA capsules were of the type

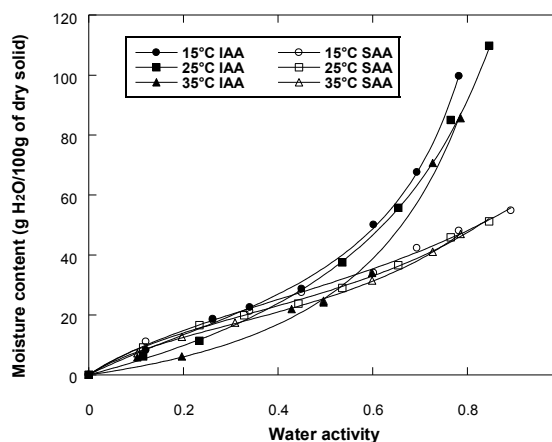


Fig. 1. Moisture adsorption isotherms of IAA and SAA products. Symbols (● □) correspond to the experimental results and continuous lines to fitted ones by using the GAB equation.

III, whereas those obtained by SAA are closer to type II. Such difference may be due to the microstructure of both systems, which induces the presence of at least two sorption mechanisms for SAA capsules, while for IAA samples, the adsorption was controlled by interaction between poly-layers. The isotherms types I and II occur when  $C > 2$ , this means that the attraction forces between the adsorbate and the adsorbent are larger than the attraction forces between adsorbate molecules in the liquid state. When the attraction forces between the adsorbate and the adsorbent are relatively small ( $C < 2$ ), isothermal type III occurs. Differences in  $M_0$  values, although somehow correlated with microstructure, did not show a regular trend with temperature since GAB model for heterogeneous materials, and mainly for those showing Type III isotherms as described by Brunauer *et al.* (1940) does not physically explain the formation of a monolayer and therefore, the evaluation of the volume of micropores must be performed to provide an explanation of the sorption mechanism.

Table 1. Estimated parameters of the GAB equation for IAA and SAA products

Product	Temperature (°C)	$M_0$ (g H <sub>2</sub> O/100g of dry solid)	$C$	$K$	$R^2$	P (%)
IAA	15	23.098	3.422	1.004	0.999	2.20
	25	42.511	1.172	0.839	0.999	3.72
	35	47.08	0.545	0.888	0.995	3.97
SAA	15	29.34	5.766	0.606	0.998	2.98
	25	22.419	6.829	0.717	0.998	3.48
	35	18.649	6.643	0.808	0.999	2.70

K values for SAA products were lower than those for IAA ones, indicating a lower number of water layers in IAA materials interacting with the solid and with other water molecules (Flores-Andrade, 2010). This was also the reason why  $M_0$  values found in this work were substantially higher than those for other products, specially those with a more homogeneous structure which, in general, exhibit Type II isotherms. Values of K close to 1 indicate a reduction of the sorption energy suggesting disruption of the multilayer to a bulk liquid-like domain (Enrione *et al.*, 2010).

### 3.2 Integral entropy ( $\Delta S_{int}$ )<sub>T</sub>

According to Hill *et al.* (1951), the minimum integral entropy can be interpreted as the moisture content required to forming a monolayer. This minimum may occur where strong bonds between the adsorbate (water) and the adsorbent (powder) exist (Nunes and Rotstein, 1991). Therefore, the maximum stability of the materials may correspond to the minimum integral entropy, since at this point the water molecules are more organized and less available to take part in deterioration reactions (Beristain and Azuara, 1990; Beristain *et al.*, 2002, Dominguez *et al.*, 2007, Pascual *et al.*, 2010). Figures 2 and 3 show the entropy changes for both types of capsules. For capsules obtained by falling drop (Fig. 2), the minimum integral entropy of water molecules occurs at moisture of 9.18 g H<sub>2</sub>O/100 g of dry solid. For capsules obtained by spraying (Fig. 3), as the water molecules are adsorbed by the powder, entropy decreases to a minimum at a moisture content of 38.01 g H<sub>2</sub>O/100 g of dry solid and this point is considered as the one of maximum stability, since water molecules have the lowest mobility at this point, and often does not match the value of  $M_0$  and  $T_g$  proposed by many authors as the point of maximum stability (Beristain and Azuara, 1990). It is also noted that as from this minimum, in SAA samples, there was a region in which the integral entropy changed little with the water content, located at the moisture range between 38.01 to 46.202 g H<sub>2</sub>O/100 g of dry solid.

Vigano *et al.* (2012) reported that an increase in the moisture content corresponding to areas of low integral entropy in pineapple pulp powders obtained by different drying methods can be related to the increase in the volume of micropores. According to the values of minimum integral entropy, SAA capsules

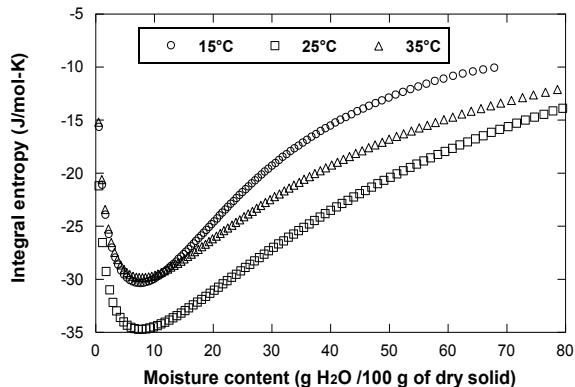


Fig. 2. Integral entropy changes of adsorbed water on IAA as a function of moisture content.

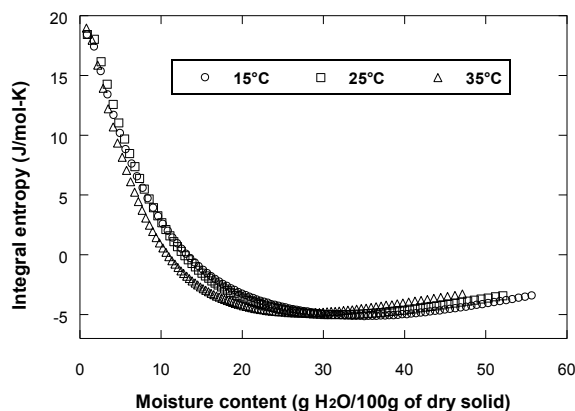


Fig. 3. Integral entropy changes of adsorbed water on SAA as a function of moisture content.

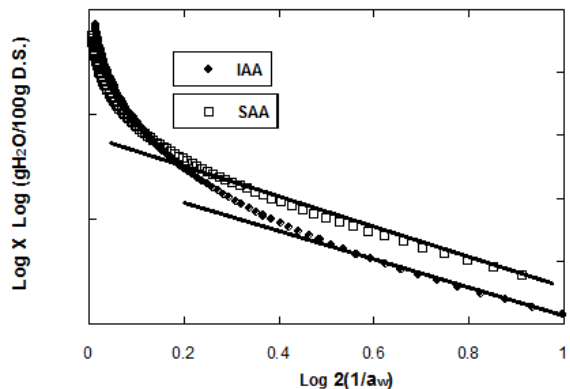


Fig. 4. Dubinin-Radushkevich graphs for water sorption in SAA and IAA products at 25°C.

Table 2. Dubinin-Radushkevich parameters for sorption isotherms of IAA and SAA products and moisture content at the minimum integral entropy at 25°C

Product	$n_0$ (g H <sub>2</sub> O/100 g of dry solid)	$R^2$	Moisture at the minimum ( $\Delta S_{int}$ ) <sub>T</sub> (g H <sub>2</sub> O/100 g dry solid)
IAA	13.80	0.998	9.18
SAA	39.89	0.944	38.01

Table 3. Ascorbic acid retention (%) during storage time at 25°C

$a_w$	SAA		IAA	
	30 days	60 days	30 days	60 days
0.115	94.75	77.37	42.78	4.22
0.234	83.47	73.13	43.95	3.91
0.443	74.25	66.07	17.64	0
0.536	73.162	63.622	14.26	0
0.654	94.103	77.948	0	0
0.765	98.20	81.95	0	0

may be stored at high moisture contents and values of  $a_w$ . These results indicate that the sorption behaviour in SAA samples is a direct consequence of changes at the microstructure level during spraying which moved the integral entropy to higher moisture and  $a_w$  values and caused a positive effect on the stability of AA during storage.

### 3.3 Volume of micropores

Sonwane and Bhatia (2000) reported that the Dubinin-Radushkevich model is widely used to calculate the micropore volume. Adsorption of gases and vapours into micropores differs from adsorption into materials with large pores or non-porous, since within the micropores water molecules are arranged homogeneously, with a decrement in the integral entropy (Azuara and Beristain, 2006). Micropores are first filled at certain  $a_w$  and, when they are full, a resistance in the mouth of the micropores is created, which reduces the sorption rate of water molecules (Azuara and Beristain, 2007). In Fig. 4, it is possible to observe that the sorption data calculated with the Dubinin-Radushkevich model for SAA were linear in nearly, the whole  $a_w$  range. For IAA capsules, sorption data were linear at low  $a_w$  values, and deviations from linearity occurred at high  $a_w$  values.

The deviation from linearity in the Dubinin-Radushkevich plots suggests complete filling of the

available volume of micropores (Fletcher and Thomas, 2000). Table 2 shows that the adsorbed moisture content corresponding to the micropore volume is greater for SAA capsules (39.89 g H<sub>2</sub>O/100 g of dry solid) than for the IAA materials (13.80 g H<sub>2</sub>O/100 g of dry solid). According to Drusch *et al.* (2009), the sorption capacity and greater stability during storage may be a consequence of a greater volume of micropores at nanoscale level. Furthermore, in Table 2, the content of adsorbed moisture corresponding to the volume of micropores is very close to the moisture content value given by the minimum integral entropy. Volume of micropores, which resulted higher for SAA capsules than for IAA materials, will influence AA retention rather than actual particle sizes obtained from both methods. High  $n_0$  values, are associated with increased AA protection. The average diameter of IAA materials was  $2.69 \pm 0.04 \mu\text{m}$ , whereas for SAA diameter found was  $3.90 \pm 0.05 \mu\text{m}$ .

### 3.4 Stability of ascorbic acid (AA) during storage

The chemical stability and its relationship with the integral entropy of the adsorbed water molecules were studied by analysing the ascorbic acid (AA) retention. According to Wilson and Shah (2007), AA-fortified products stored at room temperature have only 10-14 days shelf-life. Table 3 shows the percent

of AA retention in the samples stored at different water activities at 25 °C. Initial AA content for SAA samples, was 31140.78 mg/100g dry sample whereas for IAA materials was 31796.63 mg/100g dry sample (these values were considered as AA content at initial time of experimentation). For capsules obtained by spraying (SAA) the maximum value was 98.2% after 30 days of storage at 0.765 water activity (near to the minimum integral entropy value) and the AA was slightly decreased after 60 days. For IAA capsules, a lower retention (43.95%) than for SAA materials was achieved after 30 days of storage and was found at water activities of 0.234 (close to the value of minimum integral entropy); the retention decreased to 3.91% after 60 days. No visual microbial spoilage of materials was observed during storage.

The retention of AA for the IAA capsules as shown in Table 3 was clearly lower than retention for SAA capsules during storage over the entire range of  $a_w$  studied. This may be due to the moisture content that both types of capsules are able to adsorb before becoming thermodynamically unstable. However, the processing by freeze-drying could also have contributed to stabilise the micropores obtained by means of the coacervation step previously carried out. Freeze-drying processing has proven to be an attractive method for extending the shelf life of foods, because dehydration is carried out at temperatures below room temperature and the absence of air prevents product deterioration caused by oxidation. This method can minimize product damage due to decomposition or change in the structure, texture, appearance and flavour (Sri and Benno, 2011).

In this work, the capsules obtained by coacervation through spraying were able to maintain the stability of AA during storage at high water activities. A number of studies have been reported on the encapsulation of vitamin C, but there are few who reported stability during storage at different water activities, this is markedly important given that  $a_w$  has a great influence on the degradation and morphology of the encapsulated AA. Pulido and Beristain (2010) studied the stability of AA microencapsulated by spray drying using chitosan as wall material; they concluded that microencapsulation is an efficient method for conservation of AA and, the microcapsules obtained by these authors at low  $a_w$  (0.108) showed minimum degradation and longest shelf-life during storage, opposite to the microcapsules at high  $a_w$  (0.743), which showed the largest degradation of AA as from the first days of storage and completely destroyed morphology. Uddin *et al.* (2001) studied the effects of

processing variables on the characteristics of AA and performed four microencapsulation techniques: spray drying, thermal phase separation, solvent evaporation and dispersion-fusion. The microcapsules were stored at 38 °C and 84% RH, observing that the encapsulated AA showed slower degradation than AA in solution. Dib Taxi *et al.*, (2003) studied the conditions of the spray drying microencapsulation of camu-camu juice (*Myciaria dubia*) rich in vitamin C, using gum arabic and maltodextrin (DE10) as wall materials. The conditions for optimal retention were 15% of wall material and 150 °C inlet temperature in spray drying, resulting in vitamin C retention values ranging from 7 to 26%. Kirby *et al.* (1999) studied the stability of AA microencapsulated in liposomes during storage; results showed that the AA in solution subsisted only for 6 days at 4 °C while the AA encapsulated in liposomes stored at 4 °C presented 60% degradation after 50 days of storage.

### 3.5 Evaluation of microstructure of the capsules

Figure 5, shows images of the surface of the particles. It is noted that SAA capsules showed an irregular morphology and presented higher surface roughness than IAA capsules, at magnifications used. Moisture content corresponding to volume of micropores corresponded to water content at which the minimum integral entropy was found and which was shifted to higher values of water content for SAA materials.

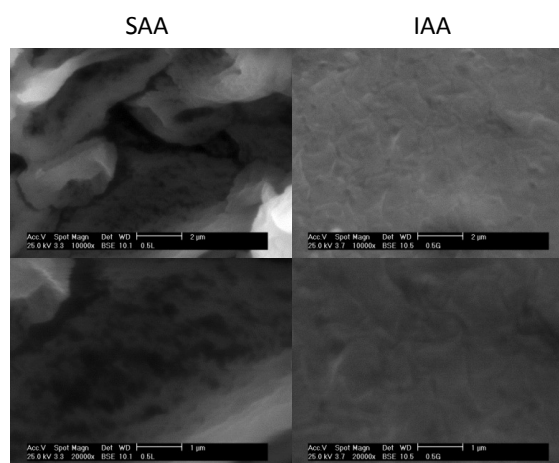


Fig. 5. Microstructure of IAA and SAA capsules at water activities corresponding to the minimum integral entropy at 25°C.



These findings allowed to understand differences in sorption behaviour of materials with the same chemical composition but subjected to different processing conditions. The microstructure of SAA capsules showed a greater volume of micropores (Table 2), which may even suggest the presence of a nanostructured matrix with reduced associated mobility of the water molecules which allowed to increase the moisture content corresponding to the minimum integral entropy for these capsules.

## Conclusions

The coacervation through spraying followed by freeze-drying, produced ascorbic acid capsules with modified microstructure, greater volume of micropores and different water sorption behaviour as compared to capsules obtained by coacervation through falling drop/freezing-drying, although the chemical composition was the same for both types of capsules. The modification in microstructure displaced the minimum integral entropy to higher moisture content and water activity values. For both types of capsules, the maximum percentages of AA retention were obtained when materials were stored at water activity values corresponding to the minimum integral entropy of the water molecules and the stability of ascorbic acid at high values of water activities was higher for SAA than for IAA capsules.

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