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DETERMINATION OF THE MINIMUM INTEGRAL ENTROPY, WATER SORPTION AND GLASS TRANSITION TEMPERATURE TO ESTABLISHING CRITICAL STORAGE CONDITIONS OF BEETROOT JUICE MICROCAPSULES BY SPRAY DRYING

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DETERMINACIÓN DE LA ENTROPÍA MÍNIMA INTEGRAL, ADSORCIÓN DE HUMEDAD Y TEMPERATURA DE TRANSICIÓN VÍTREA PARA ESTABLECER CONDICIONES CRÍTICAS DE ALMACENAMIENTO DE MICROCÁPSULAS DE JUGO DE BETABEL PRODUCIDAS MEDIANTE SECADO POR ASPERSIÓN

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Abstract

The aim of this work was to microencapsulate beetroot juice (BJ) (*Beta vulgaris* L.) by spray-drying using as protective colloid gum Arabic. The adsorption isotherms of the microcapsules and the minimum integral entropy ($\Delta S_{int})_T$ were determined at 25, 35 and 40 °C. The glass transition temperature (Tg) was measured by differential scanning calorimetry and modeled by Gordon-Taylor equation. The water contents-water activity (M-a_w) sets obtained from ($\Delta S_{int})_T$, and critical water content (CWC) and critical water activity (CWA) from the Tg were similar, being in the range of water content of 5.11-7.5 kg H_2O/100 kg d.s. and in the water activity range of 0.532-0.590. These critical storage conditions were considered as the best conditions for increase the stability of the microcapsules, where the percentage of retention Betanin in the microcapsules was higher compared with other storage conditions in the temperature and a_w range studied.

Keywords: beetroot juice, microcapsules, minimum integral entropy, glass transition temperature, critical water content, critical water activity.

Resumen

El objetivo de este trabajo fue microencapsular jugo de betabel (BJ) (*Beta vulgaris* L.) mediante secado por aspersión empleando goma Arábiga como agente encapsulante. Las isotermas de adsorción y la entropía mínima integral ($\Delta S_{int})_T$ fueron determinadas a 25, 35 y 40 °C. La temperatura de transición vítrea (Tg) se midió por calorimetría diferencial de barrido y modelada con la ecuación de Gordon-Taylor. El conjunto de valores contenido de humedad-actividad de agua (M-a_W) obtenidos a partir de la entropía mínima integral, así como el conjunto de valores de contenido de humedad crítico (CWC)-actividad de agua crítico (CWA) obtenidos a partir de la Tg resultaron ser similares, teniendo valores en el intervalo de 5.11 a 7.5 kg H_2O/100 kg d.s. para el contenido de humedad y de 0.532 a 0.590 en el intervalo de a_W. Estas condiciones críticas de almacenamiento fueron consideradas como las mejores para incrementar la estabilidad de las microcápsulas, donde el porcentaje de retención de betanina en las microcápsulas fue más alto, comparado con otras condiciones de almacenamiento en el intervalo de temperaturas y actividad de agua estudiados.

Palabras clave: jugo de betabel, microcápsulas, entropía mínima integral, temperatura de transición vítrea, contenido de humedad crítico, actividad de agua crítico.

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1   Introduction

During the past decades, there has been signi cant growing consumer interest in natural foods without stabilizers or arti cial additives. As a result, natural pigments of plant or animal origin are being used to replace arti cial colorants (Nemzer *et al*., 2011). A variety of different natural pigments have found a practical application in the coloring of food, e.g. water-soluble anthocyanins and betalains and fat-soluble carotenoids and curcuminoids.   Moreover, there is growing evidence suggesting that some natural colorants may be nutritionally important antioxidants and that their presence in the diet may reduce the risk of cardiovascular disease, cancer, and others diseases associated with ageing (Delgado-Vargas *et al*., 2000; Wootton-Beard & Ryan, 2011; Murphy *et al*., 2012).

Among the numerous natural sources of betalains, red and yellow beet, prickly pear, grain amaranth and cactus fruits are the only foods that contain these compounds.   Beetroot (*Beta vulgaris* L.) is a good source of natural antioxidant pigments (Pitalua *et al*., 2010; Wootton-Beard & Ryan, 2011), which has mainly two water soluble pigments (betalains), betanin and vulgaxanthine-I; betanin contributes ~75-95% of the total red color; while vulgaxanthine-I contributes ~95% of the yellow color (Serris & Biliaderis, 2001). Betanin is a betanidin 5-O-β-glucoside containing a phenolic and a cyclic amine group, both of which are very good electron donors, acting as antioxidants (Gliszczyńska-Świglo *et al*., 2006).

Beetroot is consumed in the form of lacto fermented juice, pickled preserves or as cooked vegetable, but the beetroot s pigments have poor stability, because there are many factors that affecting its stability during storage, exhibited at the light, and exposed to high water activities and moisture contents. Drying as a preservation method may be an alternative for a better utilization of beetroot (Figiel, 2010).

Encapsulation by spray drying has been used as a method to encapsulating avors, antioxidants and pigments, retarding the degradation of these functional ingredients, being an alternative to have a stable powder with instant properties and be a source of antioxidants for addition into food products (Murphy *et al*., 2012). Beetroot juice encapsulation by spray drying can be an alternative for a better utilization of beetroot used as a functional food in powder.

The thermodynamics of water vapour sorption provide a reliable criterion for predicting the storage stability and shelf-life of spray-dried food products (Sánchez-Sáenz *et al*., 2011).   Thermodynamic analysis of sorption needs the knowledge of isotherms behaviour as a function of temperature.   The Guggenheim-Anderson-de Boer (GAB) equation is a localized multilayer sorption and condensed lm model.   The minimum integral entropy can be interpreted as the required moisture content for forming a monolayer, where the water activity at which a dry food product is most stable (Pérez-Alonso *et al*., 2006), and where strong bonding occurs between the water (adsorbate) and the food (adsorbent) which corresponds to less water being available for chemical and spoilage reactions (Nunes & Rotstein, 1991).

The physical state of food is governed by the phase transitions of its main components, such its carbohydrates, lipids, proteins, and water.   On the other hand, it is also important to determine the changes and physical properties of spray-dried foods. In encapsulated spray-dried foods, where the food is subjected to a rapid water removal that usually results in a very hygroscopic amorphous matrix, the glass transition temperature (Tg) can be considered as a critical parameter, since collapse, stickiness, caking, agglomeration problems and re-crystallization phenomena may be avoided in the glassy state of the amorphous matrix (Roos, 1995).   In this sense, a change from a glassy to a rubbery state can occur in the spray-dried food as a consequence of an increase in the temperature or in the product water content during its storage. Depending on storage temperature, the glass transition will occur at a critical value of water content (CWC) and water activity (CWA) of the spray-dried food, which can be considered an important factor for the stability of the spray-dried food. From this point of view, it has been demonstrated that the modi ed state diagram of the amorphous phase, which includes the relationships between the spray-dried food water content, water activity and its physical state as a function of temperature, is an useful tool with which to improve product processing and storage stability (Roos, 1993; Fabra *et al*., 2009; Moraga *et al*., 2012). It allows us to predict the critical water content and water activity at which the glass transition occurs at a determined storage temperature of the spray-dried food.

The aim of this work was to study the feasibility of spray drying of red beetroot juice in four-fold: i) microencapsulate by spray-drying red beetroot juice in one protective colloid; ii) establish the most suitable storage conditions for microcapsules (water activity-temperature conditions) where the minimum integral entropy occurs; iii) related to the physical state of this

amorphous matrix with glass transition temperature; and iv) determine the betanin retention in the beetroot juice microencapsulated at different water activity-temperature conditions.

In order to contribute to optimize the technological process of spray-dried food, guaranteeing the quality and longer shelf-life of the powder product, it is hoped that the microcapsules can be potentially incorporated in dry form into the functional foods (beverages instant, cake mixes, snacks, baby foods, breakfast cereal, etc.).

2 Material and methods

2.1 Materials

The beetroots were purchased from a local market of the city of Toluca, Estado de México. Gum Arabic (*Acacia senegal*) (GA) purchased from Industria Ragar, S.A. de C.V. (Mexico City, Mexico) and was used as protective colloid. The betanin standard and all reagents used in the study were purchased from Sigma-Aldrich Quimica, S.A. de C.V. (Toluca, State of Mexico, Mexico). All the water used in the experiments was bidistilled.

2.2 Extraction and analysis of beetroot juice (BJ)

The beetroots were washed and peeled, and later the juice was extracted with a commercial juice extractor Moulinex model 140-1-03 (Naucalpan, State of Mexico, Mexico). The juice was ltered through a Tyler #9 (2000 μm) sieve in order to eliminate solids in suspension, facilitating the product s passage through the nozzle atomizer. Finally the juice was stored in a freezing chamber and later defrosted at the room temperature ($\sim$ 20 °C) right before the experiments.

2.3 Preparation of juice beetroot microcapsules by spray-drying

Gum arabic was dissolved into the BJ, to a total solid content of 30% w/w and stirred to homogeneity with an Ultra-Turrax T50 basic homogenizer (IKA-WERKE Works Inc., Wilmington, North Carolina, USA) at a speed of 5200 r.p.m. for 10 min. The solution was then fed at a rate of 40 mL/min to a Nichols/Niro spray-drier (Turbo Spray PLA, New York, USA) operated with inlet temperature of 140 ± 5 °C, outlet temperature of 80 °C± 5 °C and injecting

compressed air at 4 bar. It was no possible to spray dry the juice without adding a protective colloid, due to the high powders stickiness on the chamber wall, resulting from the presence of high sugars concentration and acids, being sucrose sugar followed by glucose and fructose and malic acids as the main constituents (Juszczak *et al.*, 2010). The spray-dried microcapsules were collected, kept in plastic bags wrapped with aluminium foil and stored in desiccators containing silica gel at room temperature. Spray-drying of each formulation was done in triplicate.

2.4 Sorption isotherms

The microcapsules were put into glass Petri dishes, taking care that the microcapsules covered completely and homogeneously the dishes surface. The dishes were then introduced into glass desiccators containing P_2O_5 as desiccant, at room temperature ($\sim$20 °C) for 3 weeks in order to reduce to a minimum water activity ($\sim$0.02) of the microcapsules. The adsorption isotherms were determined by the gravimetric method described by Lang *et al.* (1981). Approximately 1.0 ± 0.1 g of the microcapsules were put into small glass desiccators of 10 cm diameter which contained saturated solutions of different salts that provided water activities (a_W) in the range of 0.11-0.85 (Labuza *et al.*, 1985). Filter paper (Whatman No. 1) was placed above the saturated salt solutions, in a perforated plate used as support for the powders for allowing moisture transmission. Five desiccators with each type of microcapsule for each saturated solution salt were placed into forced convection drying oven Riossa model E-51 (Mexico City, Mexico) at three temperatures: 25, 35 and 40 °C. The microcapsules were weighed with an Ohaus electronic balance model AP210 (Pine Brook, New Jersey, USA) every ve days until equilibrium was achieved. Equilibrium was assumed when the difference between two consecutive weightings was less than 1 mg/g of solids. The time to reach equilibrium varied from 20 to 25 days. Water content of the humidi ed systems was determined by difference in weight after drying in a vacuum oven FELISA (Mexico City, Mexico) at 60 °C in the presence of magnesium perchlorate desiccant. The water activity was measured with an Aqualab water activity meter with temperature compensation model series 3 TE (Decagon Devices, Inc., Pullman, Washington, USA).

The Guggenheim-Anderson-De Boer (GAB) equation is a model with three parameters that have physical meaning, and is recognized as the most

versatile sorption model available for the sorption of food. It is mathematically expressed as (Carrillo-Navas *et al.*, 2011):

$$M = \frac{M_0 C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \quad (1)$$

where M is the equilibrium water content (kg water/100 kg dry solids); M_0 is the monolayer water content (kg water/ 100 kg dry solids), a_w is the water activity, and C is the Guggenheim constant, given by:

$$C = c' \exp\left(\frac{h_m - h_n}{RT}\right) \quad (2)$$

where c' is the equation constant; h_m is the total heat sorption of the rst layer; h_n is the total heat of sorption of the multilayers; R is the universal gas constant; T is the absolute temperature and K is the constant correcting properties of the multilayer molecules with respect to the bulk liquid, and given by:

$$K = k' \exp\left(\frac{h_1 - h_m}{RT}\right) \quad (3)$$

Where k' is the equation constant; h_1 is the heat of condensation of pure water.

The parameters were estimated by tting the mathematical model to the experimental data, using non-linear regression with Origin version 8.5 Scienti c Graphing and Analysis Software (OriginLab Corp., Northampton, Massachusetts, USA).

Goodness of t was evaluated using the relative percentage difference between the experimental and predicted values of water content, or mean relative deviation modulus (E), de ned by the equation (McLaughlin & Magee, 1998):

$$E = \frac{100}{N} \sum \frac{|M_i - M_{Ei}|}{M_i} \quad (4)$$

where M_i is the water content at observation i; M_{Ei} is the predicted water content at that observation and n is the number of observations. It is generally assumed that a good t is obtained when $E < 5\%$.

2.5 Thermodynamic properties

The determination of the differential and integral (enthalpy and entropy) thermodynamic properties, and the water activity-temperature conditions where the microcapsules minimum integral entropy occurred, considered as the point of maximum storage stability, was established as indicated by Pérez-Alonso *et al.*

(2006), Bonilla *et al.* (2010), Sánchez-Sáenz *et al.* (2011). These authors have provided a thorough description of the procedure followed and equations used for this purpose.

2.6 Glass transition temperature (Tg)

The glass transition temperature was determined by differential scanning calorimetry in a calorimeter TA-DSC Q1000 (TA Instruments, New Castle, DE, USA) equipped with a mechanical refrigeration system (RCS-refrigerated cooling accessory). 5 mg of microcapsules equilibrated at different water activities in the range of 0.11-0.85 and temperatures (25, 35 and 40 °C) were heated in aluminum hermetic pans. Thermal program consisted in a two cycle-scan model, with the temperature ranging from -80 at 150 °C depending on the water content at 2.5 °C/min. An empty aluminum hermetic pan was used as a reference. The DSC was calibrated for temperature using indium, and distilled water standards. The instrument was purged with nitrogen at a ow rate of 50 mL/min. The data was analyzed using Universal Analysis 2000 software, version 4.7a (TA Instruments, New Castle, USA). The glass transition temperature (Tg) was taken as the midpoint of the baseline shift in the second scan obtained in DSC. All measurements were made by triplicate.

The plasticizing effect of water on glass transition temperature was described by the Gordon-Taylor model:

$$Tg = \frac{X_s Tg_s + kX_W Tg_W}{X_s + kX_W} \quad (5)$$

$$X_s = 1 - X_w \quad (6)$$

where Tg, Tg_s, and Tg_W are glass transition temperatures of the sample (data experimental), microcapsules at zero moisture content and water, respectively; X_s is the mass fraction of solid, X_W is the mass fraction of water and k is the Gordon-Taylor model parameter, which from the thermodynamic statpoint is equivalent to the ratio of the change of component sample speci c heat at their Tg (Couchman & Karaz, 1978). Glass transition temperature of pure water was taken as $Tg_W = -135$ °C (Johari *et al.*, 1987).

The parameters for this model were estimated by tting the mathematical model to the experimental data, using non-linear regression with Origin version 8.5 Scienti c Graphing and Analysis Software (OriginLab Corp., Northampton, Massachusetts, USA). The goodness of t was evaluated by the

determination coefficient (R^2) and the mean relative deviation modulus (E). It is generally assumed that a good t is obtained when $E < 5\%$.

2.7 Scanning electron microscopy (SEM)

The microcapsules were mounted on carbon sample holders using double-side sticky tape and were observed using a JEOL JMS 7600F scanning electron microscope (Akishima, Japan) with the GB-H mode at 1 kV accelerating voltage. Micrographs at different magni cations were presented. Samples were not metalized since the microscopy equipment operates under ultra-vacuum conditions.

2.8 Betanin retention in beetroot juice microencapsulated

The water content and a_w values of the microcapsules after spray drying were determined using a moisture analyzer (Ohaus, Pine Brook, NJ, USA) and an Aqualab water activity meter with temperature compensation model series 3 TE (Decagon Devices, Inc., Pullman, Washington, USA).

The retention of betanin in beetroot juice microcapsules was determined by absorbance measurements of reconstituted microcapsules in aqueous solution with bidistilled water using a Genesys 10 UV spectrophotometer (Thermo Scienti c, Waltham, MA, USA) at 537 nm; 10 mg microcapsules were placed into 50 mL volumetric asks and solubilized with bidistilled water; the solutions were ltered and centrifuged at 10000 r.p.m. for 10 minutes with the purpose of removing gum Arabic and obtaining a homogeneous solution. A standard curve was constructed using different concentrations of betanin. The results were presented as retention of betanin percent.

To determine the stability of betanin during storage, microcapsules were stored at the range of temperatures and a_w values used for constructing the sorption isotherms. The betanin content was evaluated immediately after preparing the microcapsules and these placed in the glass desiccators at 30 and 60 days of stored. All measurements were performed in triplicate, and the average ± standard deviations (SD) was obtained.

2.9 Statistical analyses

Data were analyzed using a one way analysis of variance (ANOVA) and a Tukey s test for a statistical signi cance $P \leq 0.05$, using the SPSS Statistics 19.0 (IBM Corporation, NY, USA). All experiments were done in triplicate.

3 Results and discussion

3.1 Sorption isotherms

The experimental sorption isotherms and predictions of the GAB equations for water vapour sorption behavior at 25, 35, and 40 °C for microencapsulated beetroot juice with gum Arabic are shown in Figure 1. The water adsorption isotherms were of sigmoidal shape of type II according to Brunauer s classi cation (Brunauer *et al.*, 1938) with a more pronounced in ection point and a lower water content at equilibrium with intermediate and high water activities compared with other isotherms such as açai fruit, borojó fruit, strawberry fruit, microencapsulated (Tonon *et al.*, 2009; Mosquera *et al.*, 2010; Mosquera *et al.*, 2012). For predict the sorption properties of foods, several empiric and theoretical equations have been described, but the GAB model is the one most extensively used in foodstuffs (Serris & Biliaderis, 2001; Kurozawa *et al.*, 2009; Liang *et al.*, 2013). The experimental sorption isotherms of beetroot juice microcapsules were tted very well by the GAB model, and the parameters obtained are given in Table 1. The mean relative deviation modulus (E) values were 0.96 to 1.26 for all experimental temperatures, and all coefficients of determination R^2 were over 0.999. The GAB model is based on the monolayer moisture concept and provide the value of the monolayer water content of the material (M_0), considered as the safe moisture for dried foods during preservation, while most others models lack this parameter. The value of the beetroot juice microcapsules monolayer was within the range of 3.64 to 4.69 kg H_2O/100 kg d.s. and decreased as the temperature increased from 25 to 40 °C, these results are attributed probably to a combination of factors, such as conformation, topology and structural changes in the microcapsules. A decrease in monolayer water content can be attributed to reductions in the number of sites available for water binding as a result of physicochemical changes caused by temperature increases. High M_0 values in microcapsules may be because of enhanced sorption capacities through the swelling of hydrocolloid protective and concomitant increases in the number of polar sites (Koç *et al.*, 2010) where water may have a high affinity for hydrocolloid.

Table 1. Estimated GAB parameters for beetroot juice microcapsules.

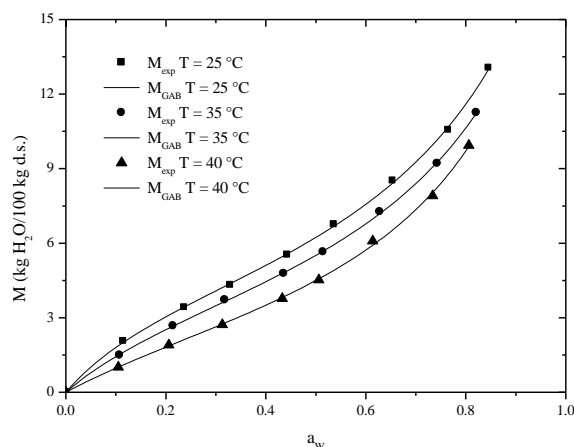| T (°C)	M_0(kg H_2O/100 kg d.s.)	C	K	R^2	$E(\%)$
25	4.69	6.42	0.787	0.999	1.07
35	4.30	5.12	0.795	0.999	0.96
40	3.64	3.53	0.839	0.999	1.26

Fig. 1. Water sorption isotherms of beetroot juice microcapsules with GA as protective colloid.

M_0 values are of particular interest, as they indicate the amount of water that is strongly adsorbed by speci c sites and they determine the physical and chemical stability of food or microbial spoilage (Pérez-Alonso *et al*., 2006; Carrillo-Navas *et al*., 2011). The values of C decreased with increasing temperature for microcapsules.

The parameter C is related to the heat of adsorption of water by the microcapsules. It is assumed that strong adsorbent-adsorbate interactions are favored at lower temperatures, resulting in an increase in C with decreasing temperature (Diosady *et al*., 1996). In this work the value of C increased with decreasing temperature, on the other hand, C values in beetroot juice microcapsules decreased with temperature, suggesting that interactions between microcapsule and water vapor are higher at 25°C than at 35°C and 40°C. Alternatively, it is possible that C lacks any physical meaning, and it is the result of mathematical compensation among parameters during the curve- tting process (Pavón-García *et al*., 2011). The values of K involve interactions between water molecules and the adsorbent (microcapsules) in the multilayer. In this study, K values were lower in microcapsules stored at 25°C, which implies that there were fewer interactions between water molecules and the microcapsules in the multilayer. Lewicki (1997)

stated that the GAB model well describes sigmoidal-type isotherms when the K values are between 0.24 and 1, besides a value of $K \ll 1$ indicates a structured state of the adsorbate in the layers adjacent to the monolayer. In beetroot juice microcapsules, the K values at the three temperatures studied fell within this range. The complex nature of adsorption behavior in food materials has been difficult to explain. For microcapsules, the process involves both adsorption and structural changes (crystalline or amorphous) of the polymer matrix because of swelling. Other factors involved in the mechanism of sorption are porosity and the speci city of water molecule attraction to the surface of hydrophilic sites in biopolymeric matrices (Viganó *et al*., 2012).

3.2 Integral entropy $(\Delta S_{int})_T$

Integral entropy describes the degree of disorder, or randomness of motion, of the water molecules. It quanti es the mobility of the adsorbed water molecules, and indicates the degree to which the water-substrate interaction exceeds that of water molecules, and therefore is a useful function by which to study the stability of the microcapsules. Figure 2 shows variations in integral entropy *vs* moisture content at the three temperatures studied. The entropy is observed to decrease to a minimum with increasing water content, and then increase with further water content increase. As the microcapsules adsorbed moisture the entropy diminished to a minimum point that is considered as that of maximum stability, because it is where the water molecules achieve a more ordered arrangement within the solid, and hence water molecules are less available to participate in spoilage reactions (Nunes & Rotstein, 1991; Pérez-Alonso *et al*., 2006). Minimum integral entropy values of the microcapsules are observed at water contents of 6.63, 6.26 and 5.59 kg H_2O/100 kg d.s. at 25, 35 and 40 °C, respectively. The initial decrease in entropy (Fig. 2) re ects an increasing restriction in the movement (loss of rotational freedom or degree of randomness) of the water molecules as the readily available sites

Table 2. Maximum stability conditions, critical values for water activity (CWA) and water content (CWC) for the beetroot juice microcapsules.

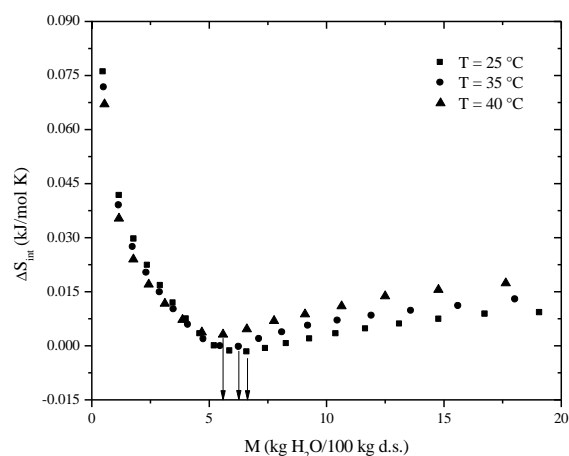| $T(°C)$	M(kg H_2O/100 kg d.s.)	a_w	CWC(kg H_2O/100 kg d.s.)	CWA
25	6.63	0.532	7.50	0.587
35	6.26	0.562	6.28	0.565
40	5.59	0.590	5.11	0.554

Fig. 2. Integral entropy as a function of water content of the beetroot juice microcapsules.

become saturated and the strongest binding sites are utilized. The minimum occurs around the monolayer moisture content, when the sorbed water becomes increasingly localized as the first layer is covered. The subsequent increase in magnitude reflects the more freely held water molecules and the formation of multi-layers. At high moisture contents the entropy will approach that of free liquid water (Benado & Rizvi, 1985). However, it can also be seen from Fig. 2 that the moisture content corresponding to the minimum integral entropy values for microcapsules to achieve maximum stability was greater than that corresponding to the GAB monolayer (Table 1). The form of the integral entropy *versus* moisture content curve is similar to that observed for passion fruit juice microcapsules (Carrillo-Navas *et al*., 2011), natural colorant from *Justicia spicigera* microencapsulated in protective colloids blends by spray-drying (Pavón-García *et al*., 2011), whey protein (Azuara-Nieto & Beristain-Guevara, 2007), and starch gels (McMinn *et al*., 2004). The conditions for maximum stability of the microcapsules determined from the minimum integral entropy are shown in Table 2. As can be appreciated, as temperature increases the water content in the microcapsules decreases and the water activity of the microcapsules increases.

3.3 Glass transition temperatures

Figure 3 shows that the relative decrease in Tg of the microcapsules due to an increase in water activity depended on the temperature storage and the protective colloid. The Tg of amorphous food matrices decreases with increasing water content owing to water plasticization effects. Sticky point, caking and collapse of microcapsules were assessed with high water content and $a_w > 0.6$, so is associated with changes of hydration and physical composition in biopolymeric matrix, leading to a modified glass transition temperature (Drusch *et al*., 2006). It has been shown that the addition of protective colloids such as maltodextrins and gum Arabic leads to a considerable increase on Tg s, confirming the efficiency in spray-dried and the improvement of the microcapsules characteristics and stability (Tonon *et al*., 2009; Ramoneda *et al*., 2011; Mosquera *et al*., 2012). The experimental data of glass transition temperatures of BJ microcapsules equilibrated at different water activities in the range of 0.11-0.85 and temperatures (25, 35 and 40 °C) were adjusted to the Gordon and Taylor model. The estimated parameters, k and Tg_s of the anhydrous microcapsules for each fitting are presented in the Table 3. The k values were similar at the three temperatures evaluated in this work (6.30-6.51). The k parameter controls the degree of curvature of the glass transition temperature that dependence on water content and can be related to the strength of the interaction between the system components (Gordon & Taylor, 1952). With respect to the Tg_s parameter, values decreased as the temperature increased, obtained values between 98.96 °C to 105.86 °C. The statistical significance among Tg *vs* water content modeled curves was analyzed, in the same way as for sorption isotherms founded values to R^2 over 0.999 and E less than 5%.

Depending on storage temperature, the glass transition will occur at critical values of water content CWC and water activity CWA of the microcapsules, which can be considered an important factor for the stability of the powder product. In order to obtain CWC and CWA values related to glass transition,

Table 3 Estimated Gordon-Taylor parameters for beetroot juice microcapsules.

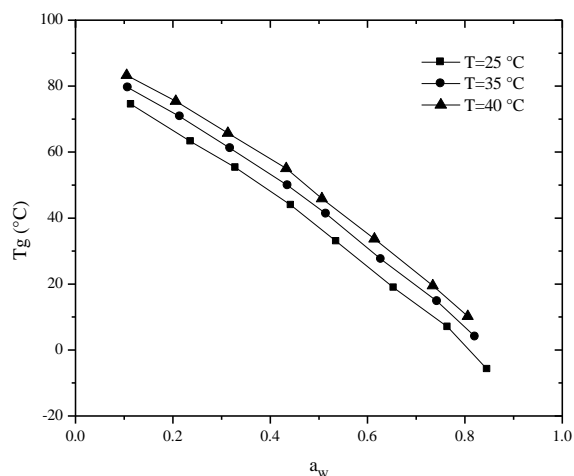| $T(°C)$	$Tg_s(°C)$	k	R^2	$E(\%)$
25	105.86	6.51	0.999	1.79
35	103.37	6.29	0.999	1.09
40	98.96	6.30	0.999	1.25

Fig. 3. Effect of a_w on glass transition temperature (Tg) of beetroot juice microcapsules.

the combined Tg-M-a_w data and the corresponding GAB and Gordon and Taylor tted models for the microcapsules were used (Fig. 4). This gure can be considered as a state diagram, showing the relationship between the microcapsule composition and its physical state as function of the temperature, which allowed us to predict the critical variables at which the glass transition occurs, at a determined storage temperature of the product (Roos, 1993). Above this temperature, the amorphous microcapsules are susceptible to deteriorative changes like collapse, stickiness and caking, resulting in quality loss. At 25 °C, the CWA and CWC for the microcapsules were 0.587 and 7.50 kg H_2O/100 kg d.s., respectively. The microcapsules stored at 35 °C showed CWA of 0.565 and CWC of 6.28 kg H_2O/100 kg d.s. The CWA and CWC values for the microcapsules stored at 40 °C were 0.554 and 5.11 kg H_2O/100 kg d.s., respectively (Table 2). The critical conditions CWA/CWC of storage for BJ microcapsules were similar to maximum stability conditions obtained for
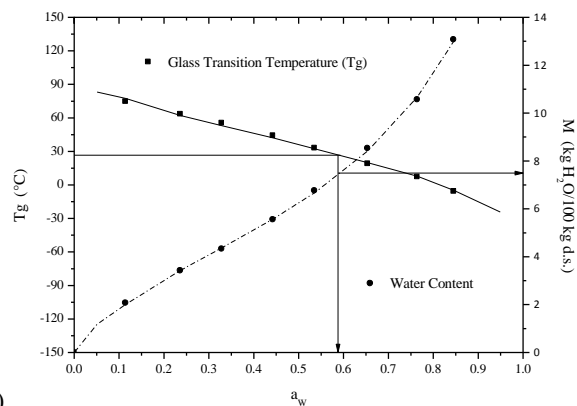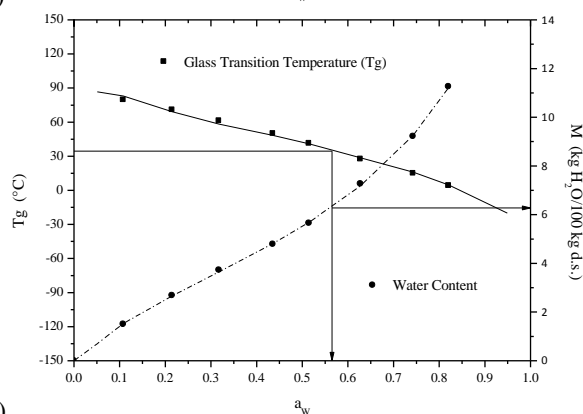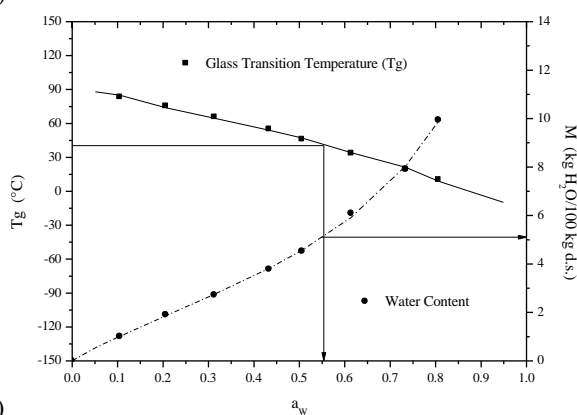
Fig. 4. Variation of glass transition temperature (solid line) and water content (dashed line) with water activity of the beetroot juice microcapsules: (a) $T = 25°C$, (b) $T = 35°C$, (c) $T = 40°C$.

minimum integral entropy, at which the microcapsules are not susceptible to deteriorative physical and chemical changes. Tonon *et al*. (2009) obtained values of CWA and CWC for microcapsules of açai juice encapsulated with different carriers agents as maltodextrin 10DE (CWA=0.574, CWC= 8.6 kg H_2O/100 kg d.s.), maltodextrin 20DE (CWA=0.535, CWC= 8.3 kg H_2O/100 kg d.s.), gum Arabic
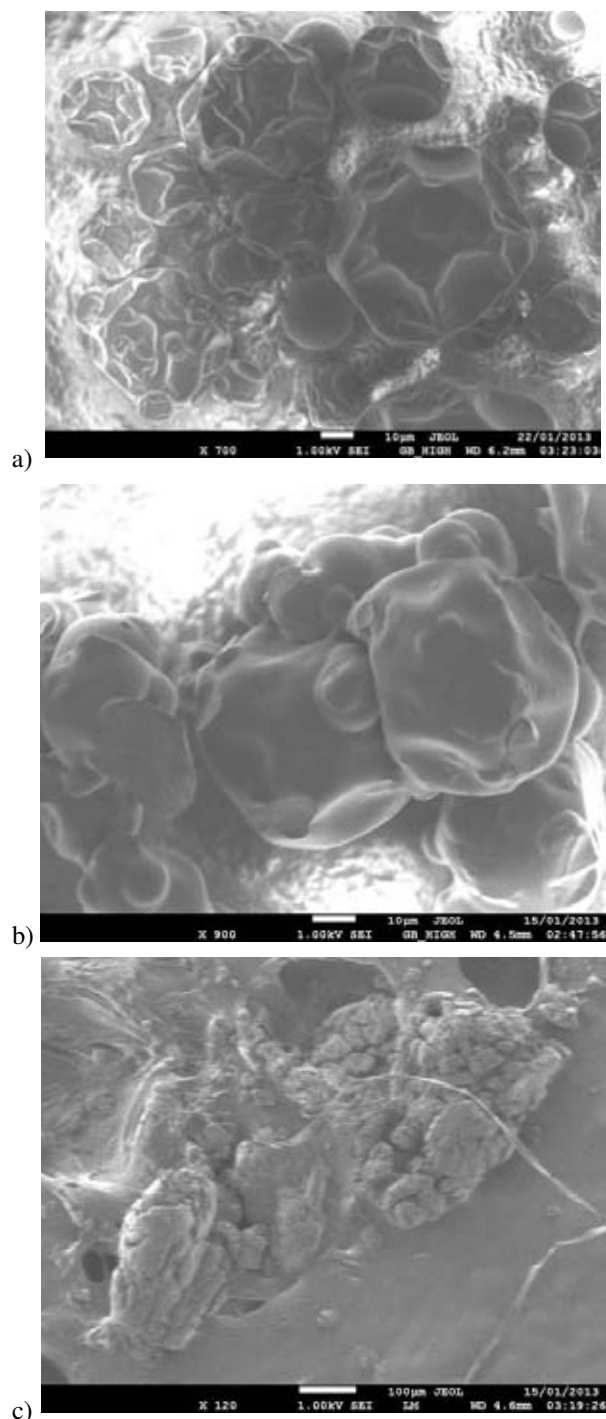
a)

b)

c)

Fig. 5. Morphology of the microcapsules stored at 35 °C: a) $a_W = 0.318$, b) $a_W = 0.515$, c) $a_W = 0.628$.

(CWA=0.571, CWC= 10.0 kg H_2O/100 kg d.s.), and tapioca starch (CWA=0.554, CWC= 6.1 kg H_2O/100 kg d.s.) when were stored at 25 °C. Mosquera *et al.*

(2012) obtained lower values of CWA (0.237-0341) for freeze-dried strawberry microcapsules with gum Arabic and maltodextrin DE16.5-19.5 stored at 20 °C.

3.4 Morphology of microcapsules by scanning electron microscopy (SEM)

The results obtained for critical water activities CWA explain the visual changes observed in the microcapsules during storage at different temperature and water activity. Independently of the storage temperature, the topology of the microcapsules stored at a_w s lower than CWA showed irregular surfaces and multiple dimples in their surfaces (Fig. 5a). The microcapsules stored approximately at CWA s values showed spheroid shapes, little s sharp surface undulations and surface integrity (Fig. 5b). The microcapsule original structure was completely lost (Fig. 5c) when the microcapsules were stored a_W s higher than CWA due to the dissolution of the protective colloid, which leads to extensive fusing of the microcapsules, producing collapse and caking.

3.5 Betanin retention in beetroot juice microencapsulated

The water content and a_w values of the microcapsules immediately after spray drying were similar and within the expected range for atomized powders (~3.5 kg H_2O/100 kg d.s. and $a_W = 0.25$) and also within the recommended values to assure microbiological stability ($a_W < 0.6$). Table 4 shows the percent of betanin retention in the microcapsules stored at different water activities at 25, 35 and 40 °C. Initial betanin content in the microcapsules was 1071.18 mg/g d.s. (this value was considered as betanin content at initial time of experimentation) that corresponds at ~92.30% of retention of betanin content in the microcapsules respect to solution inlet in spray dryer. The percent of betanin retention for microcapsules at 25 °C was clearly lower than retention for microcapsules at 35 and 40 °C during storage time over the entire range of a_w studied. The microcapsules obtained by spray drying were able to maintain the stability of betanin during storage in the range of a_w between 0.5 and 0.6, approximately. This latter range of a_w is near to CWA values and the minimum integral entropy values, where strong bonding occurs between adsorbent and adsorbate which corresponds to less water being available for chemical and spoilage reactions.

Table 4. Betanin retention in microcapsules of BJ, stored at 30 and 60 days.

T = 25 °C			T = 35 °C			T = 40 °C		
a_W	30 days	60 days	a_W	30 days	60 days	a_W	30 days	60 days
0.115	99.2	88.4	0.108	95.9	84.6	0.105	92.6	78.3
0.237	98.4	87.5	0.215	93.8	82.1	0.206	91.1	75.9
0.329	96.6	84.6	0.318	90.7	80.7	0.313	85.3	72.5
0.443	98.5	86.5	0.436	92.2	83.3	0.433	87.1	74.4
0.536	99.6	92.4	0.515	97.8	90.8	0.506	95.7	82.3
0.654	95.7	84.1	0.628	86.8	77.7	0.614	80.1	67.8
0.765	94.7	75.5	0.743	83.9	64.5	0.734	75.8	53.2
0.846	93.8	74.6	0.821	82.3	63.2	0.806	73.9	51.8

In a general way, the influence of temperature and a_w also play an important role on betanin retention of microcapsules of beetroot juice. Factors like ageing of the glassy material (microcapsule), rotational mobility and diffusion for porosity in the structure, as well as the characteristic heterogeneity of microencapsulated systems, can explain the occurrence of chemical reactions in microcapsules and like affect the stability (Slade & Levine, 1991).

Serris & Biliaderis (2001) also verified betanin degradation of water-soluble beetroot pigment stored at different temperatures (30, 40 and 50 °C) and water activities (0.23, 0.43, 0.64, 0.75 and 0.84) encapsulated in three different matrices (pullulan, maltodextrin DE5 and maltodextrin DE20). Overall, the data of the work indicated that the degradation of the betanin is highly dependent on storage conditions (temperature and a_W) where the greater degradation rates observed at intermediate a_w values and elevated temperatures (40 and 50 °C).

Conclusions

Minimum integral entropy, water sorption and glass transition temperature in conjunction allowed determine the critical storage conditions (CWA and CWC) for providing long-term physical stability and betanin retention to beetroot juice microencapsulated in gum Arabic as protective colloid. This work demonstrated that the establishment of critical storage conditions for dry products contributes to the knowledge for improving the shelf-life of microcapsules obtained by spray drying.

Acknowledgements

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