

Structural Collapse Prevents β -Carotene Loss in a Supercooled Polymeric Matrix

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The kinetics of degradation and surface color changes of β -carotene encapsulated in a polymeric matrix (PVP-40) and its relationship with physical changes (manifested as structural collapse) of the matrix were studied during storage of samples at several water activities at constant temperature. The degradation rate constants obtained decreased with an increase in the relative humidity (RH) of the storage atmosphere. β -Carotene losses were observed mainly at RHs below the glass transition temperature (T_g) of the corresponding systems, and the lower degradation constant rates were observed under conditions where the matrices were fully plasticized (i.e., rubbery) and collapsed (RH, 64 and 75%). An inverse correlation was observed between collapse and degradation rate constants. The results presented here indicated that the molecular mobility of the matrix is not rate limiting for the degradation of β -carotene. Factors such as microstructure and porosity of the polymeric matrix may be more important as modifiers of kinetic reactions. Surface color was not a sensitive indicator of β -carotene retention, because it was mostly affected by the degree of matrix hydration and collapse phenomena.

KEYWORDS: Encapsulation; β -carotene; collapse; molecular mobility; glass transition

INTRODUCTION

In recent years, the use of natural colorants has been steadily increasing, primarily because of changes in consumer preference toward more natural products known to exhibit specific functional properties. Vitamin A is the most common dietary deficiency in the world. β -Carotene theoretically possesses 50% vitamin A activity (1) while α -carotene possesses only 25% vitamin A activity (2–4). Moreover, the demand for β -carotene has increased due to reported anticancer (5, 6), free radical quencher, and other biological antioxidant activities (1, 7). However, the high degree of unsaturation in β -carotene structure renders it extremely susceptible to oxygen. The degradation appears to occur through direct oxidation without previous isomerization, and the resulting oxidation products do not have coloring properties (8). Encapsulation has found numerous applications in the food industry for coating colorants, flavors, and other sensitive or functional food ingredients in an effort to increase their shelf life as well as for the development of novel foods containing bioactive ingredients (9–12). The stability and retention of these labile biomolecules during drying and the later storage are often dependent on their encapsulation in the amorphous matrix formed during dehydration processes (13–16). However, amorphous materials undergo a structural change at the glass transition temperature (T_g). Above T_g , various time-dependent changes in the physical properties of the matrix that affect the encapsulated material can be observed (17). Most

dehydrated materials are also extremely hygroscopic, and water plasticisation decreases T_g values altering the protective effect of the initially glassy matrix (16, 18). In biological and food materials, changes in the physical structure of matrix at T_g may lead to increased permeability and diffusivity of gases that affect reaction rates and decrease the stability of encapsulated active materials (19, 20). The rate of oxidation in dried fat-containing food materials is also related to structural changes in the amorphous matrix (21, 22). Amorphous sugars are effective encapsulating agents. However, sugar crystallization promotes both the release of encapsulated lipids (22, 23) and the loss of the stabilizing effect on biomolecules such as enzymes (24). Thus, polymeric noncrystalline materials offer good alternatives as encapsulating agents.

Maltodextrins improved the shelf life of β -carotene, as compared to carrot juice spray dried alone (8, 26). The effect of the molar mass of the maltodextrins (8) or the efficiency of different drying methods on the retention of β -carotene (27) was also investigated. Polyakov et al. (28) studied the stability of inclusion complexes of carotenoids with cyclodextrins by ^1H NMR and UV–visible spectroscopy and showed that cyclodextrin protects them from reactive oxygen species.

Elizalde et al. (29) showed that the magnitude of color change, the rate of β -carotene loss, and the minimum retention of β -carotene encapsulated in a matrix of trehalose were mainly affected by the excess of moisture, above that necessary for trehalose dihydrate crystallization. In fact, when the water content is low, the available water crystallized with trehalose

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forming the dihydrate and still less water is free to liberate the encapsulated β -carotene. Once crystallization is completed, water is just involved in the liberation process; therefore, the rate of β -carotene loss was higher and the minimum retention was lower.

In noncrystalline polymeric matrices, the release of encapsulated material has been qualitatively related to structural collapse or shrinkage as a result of storage above the glass transition temperature (T_g) of the matrix (30–33). However, nonspecific studies have been performed to quantitatively relate both phenomena.

The purpose of this work was to study the kinetics of degradation and surface color change of β -carotene encapsulated in a polymeric matrix and also its relationship with physical changes (manifested as structural collapse) as a result of matrix hydration.

MATERIALS AND METHODS

Materials. Poly(vinyl pyrrolidone) of average MW 40000 (PVP-40) was obtained from Sigma Chemical Co. (St. Louis, MO). Gelatin (250 Bloom) was from Sanofi Bio-Industrias (Argentina SA). All other chemicals were of analytical grade and purchased from Mallinckrodt Chemical Works (St. Louis, MO).

Encapsulation Method. A 0.2 g suspension of β -carotene (30% w/v) was added to 350 mL of an aqueous solution of 15% PVP-40 and gelatin (0.15% w/w). Gelatin was previously dissolved in boiling water and added as an emulsifier (22). Suspensions were agitated with an omni mixer at 16000 rpm for 2 min twice. The approximate size of the emulsion oil droplets determined microscopically was 6–15 μ m. The mixture was frozen at -26 °C for 24 h to obtain ice crystals with an adequate size to give a homogeneous, porous powder and then was immersed in liquid nitrogen (-196 °C) before freeze drying to allow the highest amount of freezable water to crystallize. An Heto-Holten A/S, cooling trap model CT 110 freeze dryer (Heto Lab Equipment, Denmark) was operated at -110 °C and at a chamber pressure of 4×10^{-4} mbar. The dried emulsions were broken into powder in a mortar with a pestle and subsequently washed with hexane (high-performance liquid chromatography grade) to remove the nonencapsulated surface β -carotene until negligible absorbance was detected at 452 nm. The washed powder was further dehydrated under vacuum over $MgClO_4$.

Storage Study. Aliquots of about 2 g of the dried samples were distributed into glass vials (3 mL capacity) and exposed to atmospheres of saturated salt solutions of relative humidities (RHs) 11 (LiCl), 44 (K_2CO_3), 53 [$Mg(NO_3)_2$], 64 ($NaNO_2$), and 75% (NaCl) into evacuated desiccators at 25 °C (34). At selected times, samples were removed and analyzed for water content, surface and encapsulated β -carotene, color, and thermal transitions. All determinations were performed in triplicate, and the average value was reported.

Determination of Water Content. The water content of the equilibrated samples was determined by difference in weight before and after drying in a vacuum oven at 98 °C for 48 h. These conditions were proven to be adequate to assess constant weight after drying.

Thermal Transitions. Differential scanning calorimetry (DSC) was used to determine glass transitions temperatures (T_g) of the model systems after storage. Glass transitions were recorded as the onset temperature of the discontinuities in the curves of heat flow vs temperature. A Mettler TA 4000 analysis system was used with a TC11 TA processor and Graph Ware TA72 thermal analysis software. Temperature and heat flow in the instrument were calibrated with indium (mp 156.6 °C), lead (mp 327.5 °C), and zinc (mp 419.6 °C). Analysis in duplicate involved 40 μ L of hermetically sealed aluminum pans (Mettler) heated at 10 °C/min. An empty pan was used as a reference in all measurements.

Surface and Encapsulated β -Carotene. Surface β -carotene was determined after washing aliquots of ca. 0.7 g of the samples with 2 mL of hexane in a test tube and shaking in a vortex for 2 min. This treatment was enough to remove all of the eventual surface β -carotene present. The powder (or collapsed material) was separated from hexane by decantation, and the concentration of β -carotene in the hexane phase

was measured spectrophotometrically at 452 nm. This wavelength was found to correspond to the maximum absorbance of the spectrum from 200 to 600 nm and was in agreement with previous results reported by Lamikanra (35), Chen (36), and Desobry et al. (26). The extraction of collapsed and caked samples was more difficult than that of the powders and required special agitation with a stirrer.

Encapsulated β -carotene was determined by dispersing the remaining extracted material with 1 mL of water and shaking in a vortex for 2 min (to dissolve the encapsulated PVP matrix to liberate the encapsulated β -carotene). Then, 2 mL of hexane was added and the suspension was shaken for 2 min. The absorbance of the yellow to orange hexane fraction was measured at 452 nm. The spectroscopic measurements were performed with a UV–visible spectrophotometer (UV-1203 Shimadzu). The percent of retention of encapsulated β -carotene (%R) was determined by dividing the encapsulated concentration at any time by the total initial β -carotene concentration in the powder.

Color. A spectrophotometer Minolta 508-d (Minolta Co. Ltd., Tokyo, Japan) with an integrating sphere was used to measure the chromatic characteristics of samples during storage. A standard calibration with white and black references was performed. To perform the measurements, samples were placed in a circular cell of 2 cm diameter. Calculations were done for Illuminant D65 (corresponding to the sun light distribution spectrum) and an observer angle (determined by the visual field and the horizon line) of 2°. X , Y , and Z tristimulus values of the chromatic CIE (Commission Internationale de l'Éclairage) space were obtained. The chromatic coordinates L^* , a^* , and b^* (of the chromatic space CIE $L^*a^*b^*$) were calculated through the following equations:

$$L^* = 116(Y/Y_n)^{1/3} - 16$$

$$a^* = 500[(X/X_n)^{1/3} - (Y/Y_n)^{1/3}]$$

$$b^* = 200[(Y/Y_n)^{1/3} - (Z/Z_n)^{1/3}]$$

The $L^*a^*b^*$ color space is an international standard for color measurement developed by CIE in 1976, which is the most often used in food research (37). The color definition in the $L^*a^*b^*$ space consists of a luminance or lightness component (L^* value, ranging from 0 to 100), along with two chromatic components: The a^* component has positive values for red and negative values for green colors, and the b^* component has positive values for yellow and negative values for blue colors.

Degree of Collapse (D_c). Aliquots of 1 mL of the emulsion containing β -carotene (prepared as described above) were placed in vials (4 mL capacity, 5 mm diameter), immediately frozen using liquid nitrogen, and freeze-dried using the equipment described previously (section Encapsulation Method). These samples were transferred into evacuated desiccators at 25 °C, containing saturated salt solutions of RHs 11, 44, 53, 64, and 75%. The degree of collapse (D_c) was determined semiquantitatively by calculating the sample volume before (V_o) and after humidification (V_t), from the measured height and diameter of the samples with a caliber (38). The degree of collapse (percent) was expressed as:

$$D_c (\%) = 100 - \left(\frac{V_t}{V_o} \right) \cdot 100 = 100 - \left(\frac{h_t}{h_o} \times \frac{d_t^2}{d_o^2} \right) \cdot 100 \quad (1)$$

where V_t , h_t , and d_t are the volume, height, and diameter of the samples after humidification at time t , respectively, and V_o , h_o , and d_o are the corresponding values before storage. The D_c (%) was performed over three samples at each water activity, and the average value was reported.

Data Analysis. The kinetics of β -carotene retention, color change, and degree of collapse were modeled using a nonlinear regression program (Graph Pad Prism Version 3.1 Software). All parameters were determined with 95% confidence intervals.

RESULTS AND DISCUSSION

Physical Characteristics of the Samples. After freeze drying, the water content of the powder containing the encapsulated

Table 1. Water Content, Glass Transition Temperature, and Degree of Collapse (D_c %) of the PVP Systems Exposed to Different RHs after the Equilibration Time (Time 0) and after 40 Days of Storage at 25 °C

RH (%)	time (days)	water content ^a (% dry basis)	$T_g \pm 1$ (°C)	$D_c \pm \Delta D_c$ (%)
11	0	7.6	86	
	40	7.6	86	
44	0	14.5	50	
	40	14.5	50	
53	0	17.9	25	6 ± 1
	40	18.0	25	6 ± 1
64	0	24.1	7	20 ± 1
	40	24.2	7	35 ± 1
75	0	31.9	-9	95 ± 1
	40	32.0	-9	98 ± 2

^a The estimated relative error was 1%.

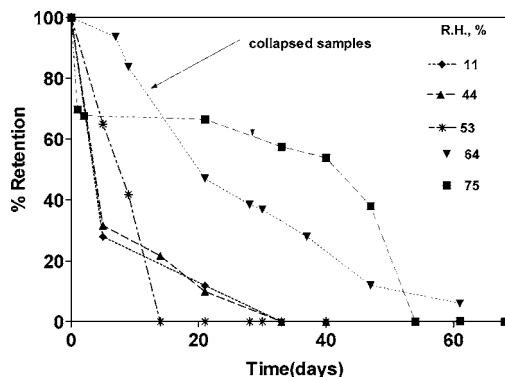
sulated β -carotene was 3.65 g water/100 g solids and its glass transition temperature was 117 ± 1 °C. Steady state mass (considered as that obtained when two measurements of sample mass taken in consecutive days differed in less than 0.0005 g) was observed after 5 days of storage over the saturated salt solutions. The samples were thus considered to be equilibrated with the corresponding atmosphere at that time, which was the initial point of the storage experiment. The steady state water contents were similar to those reported by Buera et al. (39) for PVP-40000. **Table 1** shows the water contents, T_g values, and degree of collapse (D_c) at the end of the equilibration period (time zero for storage experiments) and after 40 days of storage at 25 °C over the corresponding atmospheres. Although T_g values depend on various experimental factors (drying method, heating rate during DSC scan, etc.) and comparisons are sometimes difficult, the obtained T_g values were similar to those reported by Selim et al. (33).

The samples stored at 11 and 44% RH did not collapse during the whole storage period, while at 75% RH, the samples collapsed almost immediately, and their D_c (%) value was 95% after 5 days, which was close to the D_c value at the end of storage (98%) (**Table 1**). For the rest of the RHs studied (53 and 64%), the D_c value increased during the storage period.

β -Carotene Retention during Storage. In all analyzed cases, the concentration of surface β -carotene determined spectrophotometrically in the hexane washings was negligible. However, this did not mean that all β -carotene remained encapsulated but that β -carotene could not be detected on the surface during storage due to its fast degradation. Only the remaining fraction of encapsulated β -carotene before and after the storage experiments was analyzed in this work.

The retention of encapsulated β -carotene in PVP matrices during storage at 25 °C at all of the RHs is shown in **Figure 1**. For the samples at 11, 44, and 53% RH, the general shape of the curves was exponential with a fast approximation to a very low retention value. The same behavior was found to describe the degradation of β -carotene encapsulated in a trehalose matrix at 75% RH (29). Samples stored at 64 and 75% RH showed a completely different kinetic behavior with a low decreasing loss. These differences may be attributed to the fact that at 25 °C (storage temperature), the samples at 11, 44, and 53% RH were in the glassy state, while those stored at 64 and 75% RH were in the supercooled state (**Table 1**), and these latter showed structural collapse with a rather sticky or compacted appearance.

These different physical structures affected the retention values observed after a given storage time. In fact, for the samples stored at 11, 44, and 53% RH, the retention value was

**Figure 1.** Percent retention (% R) of β -carotene in a PVP-40 matrix, as a function of time at several RHs at 25 °C.**Table 2.** Kinetic Rate Constant for β -Carotene Degradation in a PVP-40 Matrix (k_D), with the Corresponding Standard Deviations (σ_{kD}) and Correlation Coefficients (R^2) at Different RHs at 25 °C

RH (%)	$k_D \pm \sigma_{kD}$ (days ⁻¹)	R^2
11	0.27 ± 0.05	0.987
44	0.22 ± 0.06	0.966
53	0.11 ± 0.01	0.955
64	0.01 ± 0.008	0.975
75	0.007 ± 0.02	0.623

≈ 0 after 40 days of storage, while the samples stored at 64 and 75% RH showed retention values of 30 and 50%, respectively, after the same storage time. Structural collapse affected the degradation kinetics of saffron carotenoids and of beetroot pigment encapsulated in polymeric matrices (32, 33).

The obtained curves shown in **Figure 1** were fitted according to the following model:

$$\ln \frac{R - R_\infty}{R_0 - R_\infty} = -k_D t \quad (2)$$

where R_0 and R are the percentages of β -carotene retention at zero time and time t , respectively, R_∞ is the asymptotic value to which the retention approaches at very long time, and k_D is the rate constant for β -carotene loss. This model agrees with a first-order kinetic equation for the fractional retention. Karel and Saguy (20) and Karel (19) generalized eq 2 for any property subjected to change as the result of a phase transition. Levi and Karel (31) used eq 2 to fit the fractional retention of n -propanol entrapped in carbohydrate glasses (of sucrose, and sucrose- α -D-glucopyranoside) as a function of time, and Elizalde et al. (29) described β -carotene retention encapsulated in a trehalose matrix through eq 2. The values of the constants k_D , standard deviations σ_{kD} , and the correlation coefficients between experimental values and those calculated through the model (R^2) are reported in **Table 2**. Although the correlation coefficients (R^2) between the experimental data and the predicted data through the model were higher than 0.905 ($p < 0.05$), except for the samples at 75% RH, the curve fitting was not very good, but the obtained k_D values allowed us to describe the general trends for β -carotene degradation in relation to the physical structure of the systems.

As shown in **Figure 1** and **Table 2**, the degradation rate of β -carotene in the PVP-40 system decreased as the RH increased. It is interesting to note that the kinetic rate constants of samples at 11, 44, and 53% RH (glassy state) were very similar, and they were in the order $0.11 > 0.44 > 0.53$. In these glassy systems, the retention was slightly higher when stored at 53% RH than at lower RH values, as also observed in **Figure 1**. In

the collapsed samples (those at 64 and 75% RH), the k_D values were 1 order of magnitude different between them and also 1–2 orders of magnitude lower than those for the glassy samples.

Rodríguez Huezo et al. (9) found that a maximum degradation rate of a combination of carotenoids encapsulated in hydrocolloids in spray-dried multiple emulsions occurred at 64% RH, while Serris and Biliaderis (32) and Selin et al. (33) found that the degradation rate constants for saffron and beetroot pigments encapsulated in polymeric matrices, respectively, increased with increasing RH until a value at which the samples collapsed; then, the rate constants diminished. These different kinetic responses may be related, in part, to the different water solubilities of the carotenoids as compared with that of the single nonpolar pigment β -carotene. In the more water soluble carotenoids, the rate of degradation is controlled by the access facility of the oxygen to water, while for nonpolar carotenoids it is controlled by the mobility of reactants (oxygen diffusion), which is in turn dependent on macrostructure and porosity of the matrix (32). Other factors influencing the degradation patterns are the encapsulation method and the matrix composition, which were also different to those employed in present work. Moreau and Rosemberg (40, 41) demonstrated that the permeability of wall matrix to oxygen is affected by porosity, and this determines the stability of core material. Then, physical changes in the matrices (which, as shown in the present work are determinant factors influencing kinetics and mechanisms of carotenoids degradation during storage) are expected to be different and results cannot be easily compared. The higher matrix porosity (i.e., higher volume for the same mass of solids) at lower RH allows a higher oxygen diffusion and then a higher β -carotene degradation rate constant, and this explains the kinetic responses observed in the present study (k_D diminishes as RH increases). Also, in the highly collapsed samples, the kinetic rate constants for β -carotene degradation were notably lower than those corresponding to the gassy state. The slight collapse observed at 53% RH may explain the lower K_D in this system. The structural collapse caused the disappearance or the dramatic decrease of micropores through which oxygen can enter or move in the amorphous matrix and through which all diffusion is observed (42). These observations indicated that β -carotene losses were mainly observed in the glassy state (below T_g), where it was effectively encapsulated, but the high porosity matrix allowed oxygen diffusion and then a fast β -carotene degradation. Oppositely, the lower degradation rate constants were observed under conditions at which the matrix was fully plasticized (64 and 75% RH) and the structural collapse caused the disappearance or the dramatic decrease of micropores, through which oxygen can enter or move in the matrix.

Surface Color Changes. For a given system, a decrease of the red chromatic component of the sample surface was observed during storage. The color function, luminosity L^* , was very sensitive to the surface characteristics (glassy samples had very different surface characteristics and diffusive/reflecting light properties than caked samples), and it was not representative of color changes. The chromatic coordinate b^* was also not a good indicator of β -carotene retention because it characterizes yellowness to blueness, and these colors were not dominant. The chromatic coordinate a^* (redness) was found to be the more sensitive parameter to follow surface color changes. The change in redness at a given time was calculated as the difference between the value of the chromatic coordinate a^* obtained at that time and the value at the initial time ($a^* - a_0^*$). **Figure 2** shows the changes in redness as a function of storage time. It

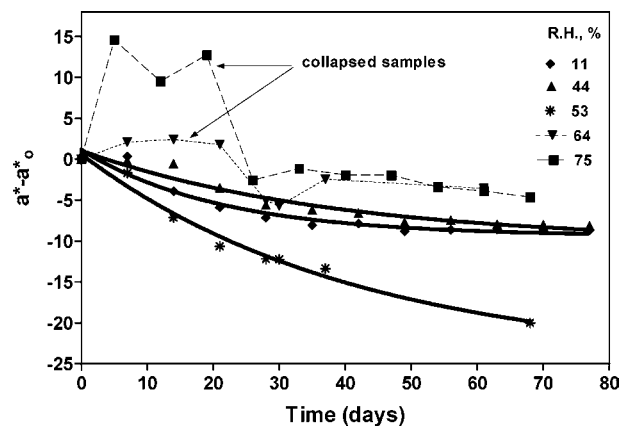


Figure 2. Change of surface color ($a^* - a_0^*$) of β -carotene encapsulated in a PVP-40 matrix as a function of time at several RHs and at 25 °C. Full lines represent predicted curves.

Table 3. Kinetic Rate Constant for Surface Color Change (k_{color}) and Color Change at Infinite Time ($a_{\infty}^* - a_0^*$) for the β -Carotene Samples Encapsulated in a PVP-40 Matrix at Different RHs at 25 °C, with the Corresponding Standard Deviations (σ) and Correlation Coefficients (R^2) between Calculated and Experimental Values

RH (%)	$(a_{\infty}^* - a_0^*) \pm \sigma_{(a_{\infty}^* - a_0^*)}$	$k_{\text{color}} \pm \sigma_{k_{\text{color}}} \text{ (days}^{-1}\text{)}$	R^2
11	-9.4 ± 0.7	0.05 ± 0.01	0.939
44	-10.2 ± 1.6	0.03 ± 0.008	0.943
53	-21 ± 7	0.03 ± 0.02	0.968

is to be noted that the negative values of $a^* - a_0^*$ indicate a loss in redness. The curves presented an initial phase of rapid change and a later phase of slower change for samples at 11, 44, and 53% RH and were fitted according to eq 3:

$$\ln \frac{a^* - a_0^*}{a_{\infty}^* - a_0^*} = -k_{\text{color}} t \quad (3)$$

where a_0^* and a^* are the chromatic coordinates at zero time and time t , respectively; a_{∞}^* is the asymptotic value at the plateau, and k_{color} is the first-order kinetic constant for color change.

Predicted color changes (full lines in **Figure 2**) showed good agreement with experimental points for the samples at 11, 44, and 52% RH. The samples stored at 64 and 75% RH were not modeled according to eq 3 because these collapsed samples presented a different behavior, and they presented a strong color as compared to glassy samples.

The surface color values at the plateau (a_{∞}^*) increased with RH while k_{color} values were approximately independent of RH (**Table 3**). Surface color changes in β -carotene encapsulated in maltodextrins followed the same trends as those obtained in the present work for the noncollapsed samples (27). The surface color changes were significantly affected by the degree of matrix hydration and collapse phenomena: The PVP-40 matrix was visually perceived as opaque and white in the glassy samples, and it became translucent in the collapsed samples, allowing us to detect the encapsulated pink pigment in the inside. Thus, the obtained k_{color} values were not representative of β -carotene retention inside the matrices because they were mostly affected by the change in optical properties (opacity) of the matrix during hydration and subsequent collapse phenomena.

Degree of Collapse. As shown in **Table 1**, the samples stored at a RH lower than 44% did not collapse. **Figure 3** shows the D_c (%) vs time for all RH > 44%. The degree of collapse (%)

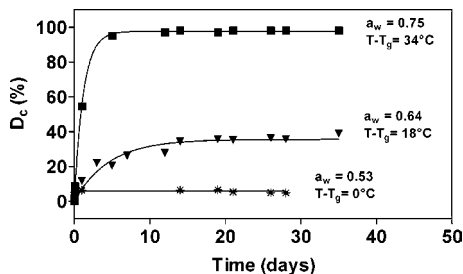


Figure 3. Percent degree of collapse (D_c %) of the PVP-40 matrix as a function of storage time at several RHs and at 25 °C. Full lines are predicted curves.

Table 4. Degree of Collapse Kinetic Constant (k_D), Asymptotic Degree of Collapse ($D_{c,max}$ %), Corresponding Standard Deviations (σ_{k_D}) and $\sigma_{D_{c,max}}$, Half-Life Time (τ), and Correlation Coefficients (R^2) for Degradation of β -Carotene Encapsulated in a Matrix of PVP-40 at Different RHs at 25 °C

RH (%)	$D_{c,max} \pm \sigma_{D_{c,max}}$	$k_c \pm \sigma_{k_c}$ (days ⁻¹)	τ (days)	R^2
64	35 ± 1	0.22 ± 0.03	3.2	0.968
75	97.5 ± 0.3	0.83 ± 0.02	0.8	0.9997

increased as the storage time increased and showed an initial phase of fast collapse followed by a phase of slow collapse, approaching an almost constant value (or plateau) at long times.

The curves of D_c vs time for RH > 44% (Figure 3) were fitted according to eq 4

$$\ln \frac{D_{c,max} - D_c}{D_{c,max}} = -k_c t \quad (4)$$

where $D_{c,max}$ and D_c are the degree of collapse at equilibrium and at time t , respectively, and k_c is the kinetic constant for collapse. The time for 50% collapse (half-life time, τ) was obtained from the following expression:

$$\tau = \frac{0.69}{k_c} \quad (5)$$

As was visually perceived, the maximum degree and rate of collapse increased with RH (Table 4 and Figure 3), where the samples at 64 and 75% RH showed a clear deformation/shrinkage, while the samples at lower RH did not present any difference from the dehydrated samples (Figure 4). As shown in Table 4, the time for 50% collapse (τ) was about 3 days at 64% RH and 1 day at 75% RH.

Despite differences in conditions used, the values calculated through eq 4 (full lines in Figure 3) fitted the experimental data with correlation coefficients (R^2) higher than 0.93 ($p < 0.05$ in all cases). For the samples stored at 53% RH, it was not possible to evaluate k_c and τ because the changes observed were lower than 6% in the storage time.

Omatete and King (30) have reported that samples of PVP-40 stored at 75% RH collapsed immediately, while at 22 and 33% RH, this phenomenon was not observed in the time frame of their experiment.

Relation between Physical State of Matrix and β -Carotene Retention. The physical changes that occurred as a result of matrix hydration were responsible for the structural change (collapse) and affected the β -carotene retention and color variations. Then, it was interesting to establish the relationship between the kinetic parameters of collapse and the β -carotene retention with RH.



Figure 4. Picture of the PVP-40 matrix samples with maximum degree of collapse ($D_{c,max}$) at several RHs at 25 °C.

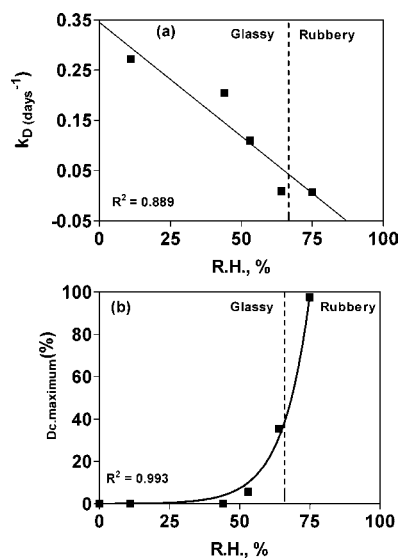


Figure 5. (a) Rate constant for β -carotene degradation (k_D) encapsulated in a PVP-40 matrix stored at 25 °C as a function of RH. (b) Maximum degree of collapse ($D_{c,max}$) of the PVP-40 matrix stored at 25 °C as a function of RH.

Figure 5a,b shows the kinetic rate of β -carotene retention (k_D) and the maximum degree of collapse ($D_{c,max}$) as function of RH. A linear relationship was observed between k_D and RH with a correlation coefficient (R^2) of 0.889. The rate constant for degradation diminished as RH increased (Figure 5a). On the other hand, the maximum degree of collapse increased exponentially with RH, and the curves predicted through nonlinear regression analysis, shown in Figure 5b, adequately represented the behavior of the experimental data ($R^2 = 0.889$).

These results suggested that the rate constant for β -carotene degradation (k_D) was affected by the degree of collapse, and Figure 6 shows that k_D exponentially decreased as the maximum collapse degree increased. The physical modifications that occurred as a result of matrix hydration were responsible for structural changes (collapse) and affected β -carotene retention and surface color of dehydrated samples.

The matrix molecular mobility was not rate limiting for β -carotene degradation, even when the encapsulating amorphous matrix was in the glassy state. Factors such as microstructure and porosity of the polymeric matrix may be more important as modifiers of kinetic reactions. The present results indicate

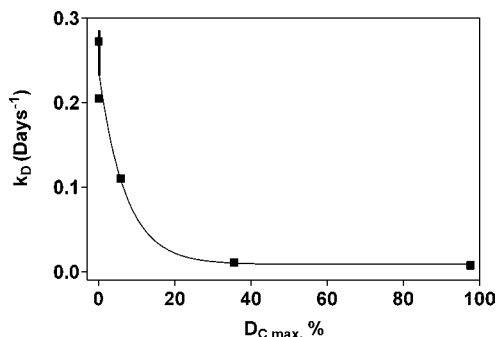


Figure 6. Relationship between the degradation rate constant (k_D) and the maximum degree of collapse ($D_{C,max}$) for β -carotene encapsulated in a PVP-40 matrix stored at 25 °C.

that matrix collapse under controlled conditions during product processing may lead to improved stability of encapsulated biomolecules. Product formulation, process, and storage may be managed through the knowledge of product dynamic properties as a function of transition temperatures and process variables (mainly water content and temperature).

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